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VOLUME 46 JUNE, 1961 NUMBER 6

TABLE OF CONTENTS

WHITTINGHILL, MAURICE, and ARCHIE CORNELIOUS ALLEN, Unchanged recovery of crossovers after X-irradiation of pupal Habrobracon 581

SANDLER, L., and YUICHIRO HIRAIZUMI, Meiotic drive in natural populations of *Drosophila melanogaster*. VII. Conditional segregation-distortion: a possible nonallelic conversion 585

SEECOF, ROBERT, Gene dosage and enzyme activities in *Drosophila melanogaster* 605

HIRAIZUMI, YUICHIRO, Negative correlation between rate of development and female fertility in *Drosophila melanogaster* 615

NUFFER, M. G., Mutation studies at the A_1 locus in maize. I. A mutable allele controlled by *Dt* 625

LONGLEY, ALBERT E., A gametophyte factor on chromosome 5 of corn . . 641

ROBERTSON, DONALD S., Linkage studies of mutants in maize with pigment deficiencies in endosperm and seedling 649

SCHMID, WERNER, The effect of carbon monoxide as a respiratory inhibitor on the production of dominant lethal mutations by X-ray in Drosophila 663

GREEN, M. M., Back mutation in *Drosophila melanogaster*. I. X-ray-induced back mutations at the yellow, scute and white loci 671

KADA, TSUNEO, C. O. DOUDNEY, and F. L. HAAS, Some biochemical factors in X-ray-induced mutation in bacteria 683

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UNCHANGED RECOVERY OF CROSSOVERS AFTER X-IRRADIATION OF PUPAL HABROBRACON

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Received January 16, 1961

ALTHOUGH the irradiation of Drosophila results in more crossover offspring, it has not been demonstrated that this effect has its origin in meiosis (WHITTINGHILL 1955) rather than in gonial exchange. Hence it is appropriate to irradiate specimens or stages which may have only oögonia rather than the mixture of gonia and gametocytes carried by adults. Previous experiments (CLARK 1943) showed that Habrobracon had no significant variation in recombination percentage among offspring from females of increasing age and no change following exposure of adults either to temperature extremes or to a 560r dose of X-rays. In these three respects Habrobracon differs from Drosophila. Possible conclusions are that the chromosomes of Habrobracon require stronger environmental assaults to change crossing over, that crossovers are formed and then lost, or that the wasps lack some stages of cells in the germ line which are both present and responsive in Drosophila.

We wished to investigate whether heavy doses of irradiation given before meiosis began would produce crossovers in the Drosophila pattern. The four egg strings (ovarioles) of Habrobracon contain no oöcytes until a late pupal stage. Irradiation of oögonial cells could be made on the "white pupa" stage on the sixth postzygotic day. Crossover sons could be distinguished from those mutation sons which might mimic some crossover phenotypes if we could use as many as four linked markers, as had been done by CLARK. The mutants black (*bl*), lemon (*le*), cantaloup-25 (*c*) and honey (*ho*) have been pictured in WHITING (1939) and offer little or no confusion in classification.

Stocks and all crosses, except those to be irradiated, were made by placing three pairs of the parasitic wasps in shell vials (15 × 75 mm) with six well-developed prestung moth larvae (*Ephestia kueniella* Zeller). All wasp cultures were kept in an incubator at an optimum temperature of 27±1°C, except when transfers were made in the laboratory at 25°C.

The parents of the wasps to be treated with X-rays were set in lucite boxes (4.7 × 4.7 × 2 cm) with five prestung caterpillars each. Seven days after making sets the lucite boxes were opened and the white pupae were irradiated *in situ*. The wasps were divided into four groups: a control group, which remained just outside the radiation therapy room, and three groups, which started receiving irradiation simultaneously and were removed after having received 1000r, 2000r or 3000r. X-rays were delivered at the rate of 341r per minute with a Picker deep therapy unit (located in the Department of Radiology of the North Carolina

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TABLE 1

Percentage of crossovers in three regions (bl–le, le–c, c–ho) *after different dosages of X-rays to immature Habrobracon*

Dosage r	Females tested	Offspring	Regions 1 (*bl–le*)	2 (*le–c*)	3 (*c–ho*)
0	9	1,398	27.54±1.20	8.65±0.76	24.89±1.16
1,000	9	2,025	27.64±0.99	10.30±0.67	25.07±0.96
2,000	9	1,570	27.77±1.09	8.14±0.68	26.03±1.10
3,000	11	330	25.44±2.38	5.45±1.20	31.80±2.56

Memorial Hospital). A current of 20 ma and a voltage of 220 kv peak was used. The target to surface distance was 10 cm.

The F_1 females were isolated as virgins to produce haploid sons. Each female was set individually in a sterile shell vial with four prestung caterpillars. The females were transferred every two days to fresh caterpillars through the 18th day and according to fertility afterwards. Crossovers were detected among their haploid offspring. Parent wasps and their cultures were coded to avoid bias in the care, transfer and classification of treated and untreated material.

RESULTS AND DISCUSSION

The frequencies of recombination (with standard errors) are presented in Table 1. Crossovers were especially uniform in region 1, agreed well in region 3, and varied randomly with respect to dosage in region 2. Coincidence values were .96 for 352/366 region 1–3 doubles, .88 for 111/125.9 region 1–2 doubles and only .13 for 14/107.6 region 2–3 double crossovers. All four treatment groups agreed in having few or no double crossovers in the adjacent *le–c–ho* regions. CLARK, using Drosophila as a model, suggested that the spindle attachment is near lemon. Our data would further indicate that it is to the right of lemon in the *le–c* region.

The total number of offspring was tabulated for each group by the successive cultures to look for periods of high lethality, if any. From Table 1 it is clear that only the 3000r group showed reduced fertility, whereas the other irradiated females were more fertile than the controls. Although the controls at first produced slightly more sons than did any treated group, after eight days of egg laying the 1000r and 2000r groups "overcompensated" and produced substantially more offspring than did the controls. This seems to indicate a stimulating effect of small irradiation doses as well as the sterilizing effect of large doses. However, there seem to have been no gaps other than in the first six days such as might have been left by crossover chromosomes containing lethal duplications or deficiencies arising either before or during meiosis.

Several explanations of the contrasts between Habrobracon linkage studies and the classic Drosophila crossing over experiments of MULLER (1925) and others may be offered: (1) formation of "crossovers" each having a deficiency or a duplication (pseudocrossovers) large enough to kill every Habrobracon carrying

one, but not always lethal in Drosophila of crossover phenotypes; (2) radioresistance of individual chromosomes in the wasp, less resistance in the fly; or (3) lack of a proper target for crossing over in the Hymenoptera but presence of a competent target, somatic pairing, in many Dipterans.

The formation and variable loss of pseudocrossovers does not seem to be the explanation of the differences. It is unlikely that *all* exchange chromosomes of Habrobracon would be lethal; a few small duplications in otherwise crossover-type chromosomes should survive. However, we have found no small increases of recombination frequency attended by large decreases in total fertility in the wasp experiments. In Drosophila the recovered crossover chromosomes from irradiated male heterozygotes have shown very little lethality in homozygous individuals and no consistent alteration in subsequent crossing over in the region of induced exchange (WHITTINGHILL 1955). From irradiated females also the frequency of new recessive lethals in Drosophila crossover chromosomes is not significantly above that of noncrossovers (WHITTINGHILL, unpublished). Unless there is evidence of decreased production of haploid wasps and evidence of great lethality of flies homozygous for crossovers from early eggs, an important role for pseudocrossovers seems lacking.

Alternatively there may be a great chromosomal difference in ability to exchange by crossing over in Habrobracon as compared with Drosophila. There is little direct evidence for this in the literature, but a related process—breakability of chromosomes—has been studied, and X-rays have been found to cause visible breakage in a wide variety of plant and animal chromosomes. Thus we may tentatively conclude that although the chromosomes of Habrobracon may not be radioresistant to breakage they appear resistant to induced exchanges of the crossover type. Since a relation of crossing over to breakage has not been established, it seems unwise to conclude that the generic differences lie in the chromosome fragility.

The best explanation of the three considered would seem to lie in the observed prevalence of somatic pairing of chromosomes in Drosophila and other Diptera, an arrangement apparently lacking in the Hymenoptera. The irradiation of gonia in Drosophila (WHITTINGHILL and DAVIS 1961) and the chemical treatment of Drosophila larvae (WHITTINGHILL and LEWIS 1961) have been followed by induced crossovers from treated males and by a temporary increase in crossovers from Drosophila females. In experiments of WHITTINGHILL (1955) and others the increase in recombinations from Drosophila females has continued for 18 days in some families. Yet Habrobracon showed no changes when irradiated as heavily and bred even longer. Furthermore the wasps showed no female to female variability in recombination such as Drosophila shows, and this variability would be expected to be accentuated in specimens with only four sources of gonia as compared to Drosophila's 30 ovarioles, but such individual variations were not found among either irradiated or control wasps. Our experiments made no test of recombination induced directly in meiotic stages, but the earlier experiments of CLARK showed no effect of X-rays nor of several temperatures upon adult

Habrobracon. Therefore we may assume a stability for the meiotic mechanism of Habrobracon and perhaps also for Drosophila until further experiments may narrow down the origin of variability.

SUMMARY

1. Recombination among haploid sons from Habrobracon mothers heterozygous for four linked markers showed no significant variation (a) between females, nor (b) within age groups from ages 2–38 days, nor (c) following X-radiation doses of 1000r, 2000r or 3000r to the white pupal stage. These observations extend those made by CLARK after treating adult wasps.

2. Total fertility was increased among lower dosage females and was drastically reduced in the 3000r group.

3. The constancy of recombination in the wasp was attributed to the lack of synaptic chromosomes in gonial cells rather than to radioresistance of chromosomes or to complete selection against pseudocrossovers in haploid larvae and pupae.

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We are grateful to DR. A. R. WHITING for her interest and advice in locating the necessary mutants, to DR. LEO LACHANCE for supplying the four separate stocks and giving much technical advice, and to DR. HOWARD ERDMAN for unpublished data on sublethal doses of X-rays.

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MEIOTIC DRIVE IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER. VII. CONDITIONAL SEGREGATION-DISTORTION: A POSSIBLE NONALLELIC CONVERSION[1,2]

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MALES, heterozygous for the *Segregation-distorter* (*SD*) allele (located in or near the centromeric heterochromatin of the right arm of chromosome II) and a standard tester second chromosome, regularly produce a preponderance of functional *SD*-bearing sperm (SANDLER, HIRAIZUMI and SANDLER 1959). Segregation in heterozygous *SD* females, on the other hand, is always normal. It has been found, however, that, for certain *SD* lines, if the *SD*-bearing chromosome is inherited from the female parent, then, in a fraction of F_1 male sibships, only one half of the heterozygous *SD* sons exhibit the phenomenon of segregation-distortion; in the other half of the sons, segregation is normal. In the following generation, all of the males exhibit abnormal ratios irrespective of whether or not their father showed an abnormal ratio. Those females that produce sons, only one half of which distort, are referred to as *conditioned;* the phenomenon is termed *conditional distortion* (SANDLER and HIRAIZUMI 1959).

The genetic basis of the phenomenon of conditional distortion has now been elucidated. The system operates as follows. The X chromosome, in a male carrying *SD*, may become induced into a specific modifier of *SD* such that in the succeeding generations the newly induced X chromosome suppresses segregation-distortion in appropriate tester lines. Thus, if *SD*-bearing males are mated to normal females, the female progeny have two kinds of X chromosomes, a normal X from the mother and a changed X from the father. Such a female is said to be conditioned. Her *SD* sons will be of two types. Those that receive the normal X will be distorters; those that receive the changed X will produce only normal segregation ratios. Both kinds of males, when crossed to normal females, produce, in the next generation, sons all of which receive an unchanged X chromosome from the female parent and therefore, if *SD*, produce abnormal ratios.

The evidence for this interpretation, and other questions related to the phenomenon of conditional distortion is presented below.

A demonstration of conditional distortion: The *SD* line that was used to investigate the question of conditional distortion is the *SD*-bearing chromosome, *R(SD)-4*. This chromosome is a recombinant between *SD-72* (a line originally

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collected in nature and containing an inversion in IIR) and the standard *cn bw* tester chromosome (a normal chromosome II carrying the mutants cinnabar and brown). The exchange giving rise to *R(SD)-4* took place proximal to the inversion in *SD-72*, so that *R(SD)-4* is inversion free and is marked by the mutant allele *bw*. In addition, *R(SD)-4* has been backcrossed to the standard *cn bw* line for many generations (passing *R(SD)-4*, however, through males only) so that the genetic background is standardized. Crosses of *R(SD)-4/cn bw* males by *cn bw* females gives rise, in every generation, to the standard semistable distribution of k values (SANDLER and HIRAIZUMI 1960). The parameter k is defined as the proportion of functional *SD*-bearing gametes produced by a heterozygote.

When, however, *R(SD)-4/cn bw* males are crossed to *In(2LR)Cy/cn bw* females (the Curly chromosome being a standard second chromosome balancer and, in this stock, having been backcrossed for many generations to the standard *cn bw* stock to insure uniformity of genetic background) and the *R(SD)-4/In(2LR)Cy* female progeny crossed individually to *cn bw* males, two types of *R(SD)-4/cn bw* male sibships are recovered—those in which all of the males give distorted ratios (the mother being referred to as "unconditioned") and those in which only half of the sons distort (the mothers then being termed "conditioned"). The results of such a series of matings are shown in Figure 1. Histogram A shows the standard semistable distribution for *R(SD)-4* inherited through males; histogram B shows the distribution of k values of the sons of conditioned females (the mean k value slightly higher than 0.50 exhibited by the nondistorting males is almost certainly accounted for by the relative inviability of flies homozygous for the mutants *cn* and *bw*); histogram C shows the distribution of k values of the sons of unconditioned females; and histogram D shows the distribution of k values of the sons of those males showing no distortion in histogram B. It can be seen (1) that unconditioned females produce male progeny apparently not different from males receiving this *SD*-bearing chromosome from a male parent (that is, histograms A and C are very similar); (2) that the distorting males of conditioned females give a completely standard distribution (the right side of histogram B is similar to histograms A and C); (3) that recovery, in the next generation, is complete (histogram D is similar to A, C, and the high side of histogram B); and (4) that induction does not always occur even when it obviously could.

The formal basis of conditional distortion: The phenomenon of conditional distortion does not occur if the female through which *SD* is passed carries a compound X chromosome so that the X in the male progeny is patroclinous. The compound X chromosome used in these studies is a reversed acrocentric compound marked by the recessives, yellow and forked (symbol: *y f:=*). Thus, single *R(SD)-4/cn bw* males were doubly mated both to free-X females carrying *In(2LR)Cy/cn bw* and to *y f:=*/Y females also carrying *In(2LR)Cy/cn bw*. These compound-X-bearing females have been backcrossed to the standard *cn bw* stock for some generations so that the genetic background is again from the

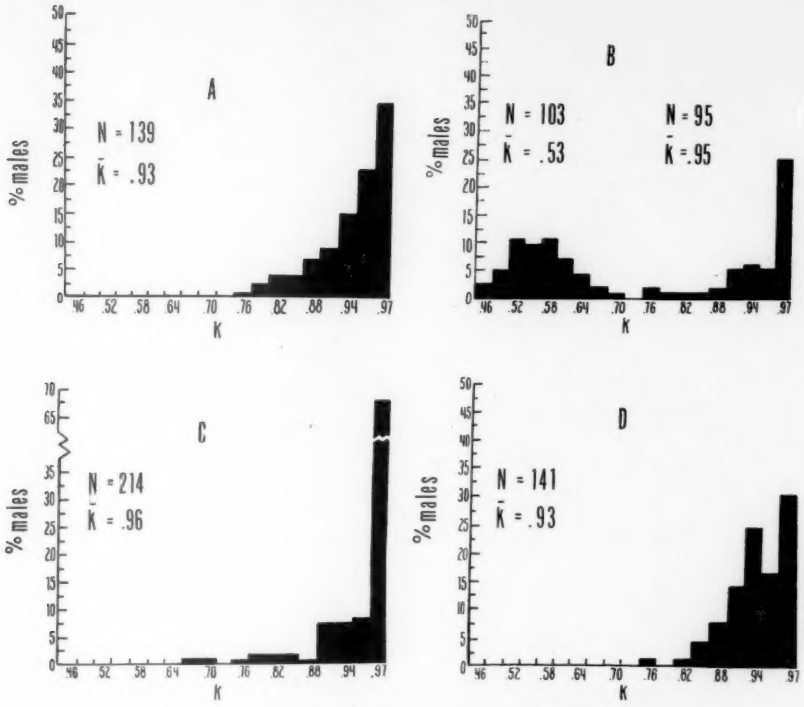

FIGURE 1.—A demonstration of the phenomenon of conditional segregation-distortion. Histogram A = *R(SD)-4* inherited from male parents; B = *R(SD)-4* inherited from conditioned female parents; C = *R(SD)-4* inherited from unconditioned female parents; D = *R(SD)-4* sons of the nondistorting males recorded in histogram B. N = total males tested; $\bar{k}$ = unweighted mean k value.

standard stock. From each mating, *In(2LR)Cy/R(SD)-4* females were collected and crossed to *cn bw* males. From these crosses, *R(SD)-4/cn bw* male sibships were obtained from individual females and tested for segregation-distortion. The results of these experiments are given in Figure 2.

Histogram A shows the k value distribution of the sons of those males mated to normal, free X females which were unconditioned. The results from the corresponding crosses to compound X-bearing females are given in histogram B. The results from conditioned females are shown in histogram C (free X) and the corresponding results from compound X females in histogram D.

It is clear from these data that, while the free X daughters of these males may be either conditioned or unconditioned, the compound X-bearing daughters produce only distorting sons. Formally, then, the phenomenon of conditional distortion is suppressed in compound X and Y-bearing females. It may be noted that

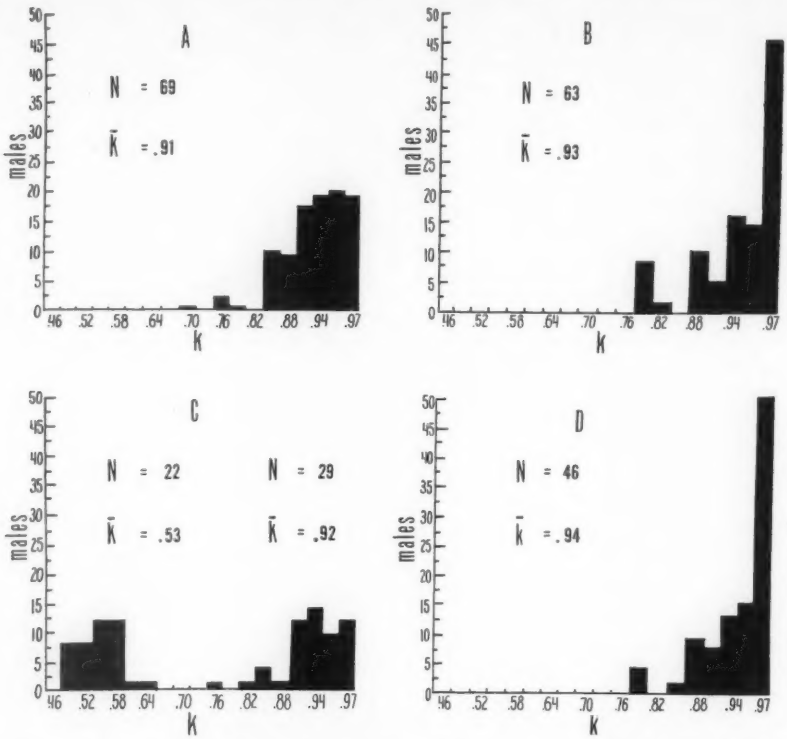

FIGURE 2.—The suppression of conditional distortion in compound X and Y-bearing females. Histogram A = the sons of unconditioned free X females; B = the sons of compound X-bearing females carrying the same *SD*-bearing chromosomes as in A; C = the sons of conditioned free X females; D = the sons of compound X-bearing females carrying the same *SD*-bearing chromosome as in C.

this same result has been obtained utilizing a simple attached-X rather than the reversed acrocentric compound X.

This suppression of conditional distortion in compound X females and the equality of distorting and nondistorting sons from conditioned free X females (Figure 1; histogram B) suggested that the X chromosome that the female inherited from her father with the *SD*-bearing chromosome II might be changed and that those of her sons that receive the changed X (i.e. the X chromosome from the maternal grandfather) do not distort. Thus it may be imagined that in an *SD* male, the X chromosome is induced to become a specific modifier of *SD* such that in succeeding generations it inhibits segregation-distortion.

In order to test this hypothesis directly, the following experiments were performed. First, individual *R(SD)-4/cn bw* males were doubly mated both to

y f:=/Y; *cn bw/cn bw* females and to free X *cn bw/cn bw* females and the resulting *R(SD)-4/cn bw* sons from each cross tested for distortion by crossing them to free X females. The results of these experiments are given in Figure 3.

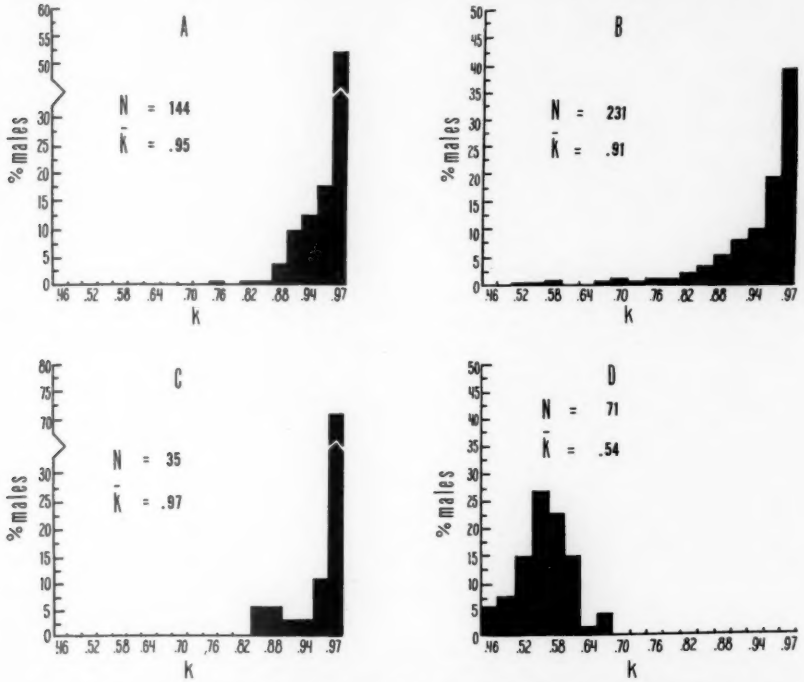

FIGURE 3.—Conditional distortion through males. Histogram A = the sons of *R(SD)-4* fathers mated to free X females; B = the sons of these same fathers mated to compound X and Y-bearing females; C = the sons of *R(SD)-4* fathers mated to free X females; D = the sons of these same fathers mated to compound X and Y-bearing females.

Here, histograms A and C are the *k* value distributions of the sons of the cross to free X females. Histograms B and D show the two kinds of results from crosses to compound X-bearing females. Histogram A is the control for histogram B; histogram C is the control for the results shown in histogram D.

It can be seen that all of the males from the free X female cross distort (which is, of course, expected since this is the standard mating by which the *SD* stock is kept) while from the compound X female set, the sibships are either all distorting or else all nondistorting. The sons of those males showing no distortion in this generation were also tested and, in the next generation, they all distorted normally. Thus recovery is complete in this case also. It seems clear, therefore, that the phenomenon of conditional distortion does not require that *SD* pass

through a female for the reason that in these experiments the *SD*-bearing chromosome was inherited only through males throughout the experiment.

More important, however, is the fact that males receiving their X chromosome from their *SD*-bearing male parent exhibit conditional distortion—but since an entire male sibship inherits the same X chromosome, the sibships are nonsegregating.

It is interesting, nevertheless, to note that even in unconditioned sibships from males mated to XX/Y females, there are occasional low k values (about 15 to 20 percent) which are not found in the free X controls. It seems, therefore, that some changed X's are produced in sibships containing predominantly uninduced X chromosomes. This point will be considered again below.

A second set of experiments designed to test the hypothesis that changed X's are responsible for the nondistorting males of conditioned females was as follows. Single *R(SD)-4/cn bw* males were crossed with Muller-5/+; *cn bw/In(2LR)Cy* females. The Muller-5 chromosome is a standard X chromosome balancer marked by the dominant mutant Bar. In this stock, this chromosome had been repeatedly backcrossed to the standard *cn bw* stock to insure genetic uniformity. It has been found that Muller-5 carries a suppressor of distortion that is unrelated to the present investigation. The nature of modifiers of segregation-distortion will be reported on in detail in a subsequent report. Because of this modifier, however, it is not possible to determine whether a Muller-5 chromosome has become changed by virtue of being with *SD*. However, the Muller-5 chromosome is used solely to provide genetic markers for identifying the paternal and maternal X chromosomes in conditioned females, so its behavior with respect to *SD* is irrelevant. Thus from the cross, *R(SD)-4/cn bw* males by Muller-5/+; *In(2LR)Cy/cn bw* females, Muller-5/+; *R(SD)-4/In(2LR)Cy* females were collected and crossed individually to *cn bw* males. From among these progeny, +/Y; *R(SD)-4/cn bw* males were collected and tested for distortion by crossing to free X *cn bw* females. The results of these crosses are given in Figure 4.

It can be seen that such male sibships, which contain only the one X chromosome ultimately derived from the *SD*-bearing maternal grandfather contain either all distorting (histogram U for unconditioned) or all nondistorting (histogram C for conditioned) males showing that it is indeed the grandpaternal X chromosome that is always present in the nondistorting sons of conditioned females. It should be mentioned that again, in the next generation all the males distort.

From these results taken together then, the hypothesis that in *SD*-carrying males the X chromosome may become a specific inhibitor of segregation-distortion in subsequent generations, may be taken as demonstrated.

We may note an additional point about the phenomenon of conditional segregation-distortion. It seems clear that the change in the X chromosome is *specific induction*. Three arguments effectively eliminate the possibility that the results reported are the consequence of segregating modifiers of segregation-distortion. First, the background genotype, including the X chromosomes, is uniformly

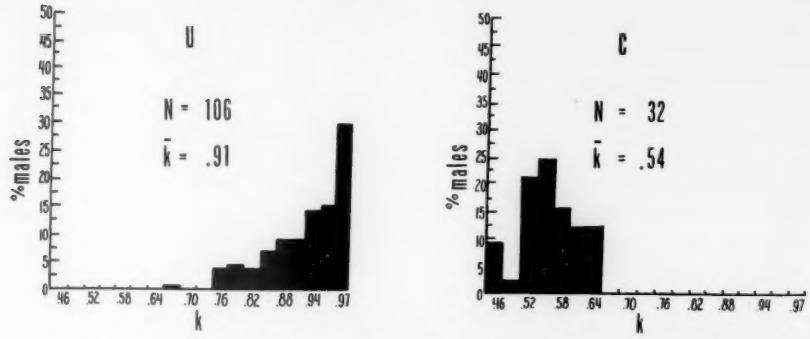

FIGURE 4.—The effect of the grandpaternal X chromosome inherited through a female. Histogram U = those grandpaternal X chromosomes associated with unconditioned behavior; C = those grandpaternal X chromosomes associated with conditioned behavior.

from the *cn bw* stock. Thus, if there were modifiers of some sort segregating in the *cn bw* stock that suppress distortion, these modifiers would express themselves, in the same way irrespective of whether *SD* is inherited from the male or female parent. Second, those heterozygous *SD* males that transmit the changed X (i.e. that produce conditioned females) are themselves distorters indicating that they do not carry any suppressors of distortion. Finally, the complete recovery of the ability to distort after crossing to free X females eliminates the possibility of any segregating modifiers on the Y chromosome, or indeed on any chromosomes other than the X. It therefore seems inescapable that in an *SD* male, there is specific induction of a modifier of distortion on the X chromosome.

Additional observations on conditional distortion: It has been found previously that the *SD* region is complex (SANDLER and HIRAIZUMI 1960a). It may be thought of as consisting of two separable elements: (1) the *SD* locus itself that causes an effective break in its homolog, which break results in the nonfunctioning of SD^+-bearing spermatocytes or spermatozoa and thus leads to abnormal genetic ratios, and (2) an *Activator* of *SD*, *Ac(SD)*, that is necessary, in coupling with *SD*, in order that *SD* operate. The *SD Ac(SD)* complex apparently resides in a small chromosomal aberration that is immune to *SD* action, resulting in complete insensitivity of *SD*-bearing chromosomes to the action of *SD* and thus also essentially "directing" the *SD* chromosome to break its homolog instead of itself.

It is of interest, in this connection, to inquire as to which element or elements are necessary in order that the changed X chromosome be generated. Accordingly, males carrying *cn bw* and a chromosome bearing the *SD* subelement but not *Ac(SD)* and only a small part of the associated aberration, SD^{in}, (SANDLER and HIRAIZUMI 1960a) were crossed to *cn bw/In(2L)Cy cn bw* (*In(2L)Cy* carries only the left arm *Cy* inversion and is distortable). SD^{in}*/In(2L)Cy cn bw* female progeny were crossed to *R(SD)-4/cn bw* males and the *R(SD)-4/In(2L)-Cy cn bw* male progeny were tested. In five conditioned families there were pro-

duced 38 males showing the usual amount of distortion and 45 males showing approximately 1:1 segregation ratios. Therefore, it can be concluded that the *SD* sub-element alone can generate changed X chromosomes.

An experiment has been performed for the purpose of defining the difference between a conditioned and an unconditioned female. The question to be answered is whether an unconditioned female is one that has not received a changed X chromosome from her father or whether such a female is one that has received a changed X, but also carries an unresponsive *SD* chromosome so that the induced X cannot function. In general, then, we may inquire whether the difference between the conditioned and unconditioned state is defined by the state of the X chromosome or of *SD*.

To answer this question, the following experiment was performed utilizing in addition to stocks already mentioned, *SD-5* (an original *SD* line collected in nature that contains two inversions and a lethal in IIR). The two inversions prohibit crossing over in the right arm, between *SD-5* and its homolog (SANDLER, HIRAIZUMI and SANDLER 1959). Single *R(SD)-4/cn bw* males were doubly mated both to *y f*:=/Y; *cn bw/cn bw* females and to *SD-5/cn bw* free X females. From each of these matings, *R(SD)-4/cn bw* males were collected and tested for segregation-distortion. The results of these experiments are given in Figure 5.

Histograms A and C show the *k* value distributions of the sons of *R(SD)-4* males crossed to compound X-bearing females. Only the sons of males that produced distorting progenies were chosen, although the other type of sibship was present. Histogram B shows the distribution of *k* values of the sons of the same males used in histogram A, but mated, in this case, to *SD-5/cn bw* free X females. Histogram D corresponds in the same way to histogram C.

From these results, it is clear that a conditioned female is defined by the possession of an X chromosome that is changed. This is so for the reason that even when the *SD*-bearing chromosome and the X chromosome together in a male exhibit the unconditioned behavior (histograms A and C), the same *SD* exhibits the typical conditioned (or else unconditioned) behavior in the presence of an X chromosome from a different *SD* line.

These experiments, it might be mentioned, also show that a modified X chromosome can inhibit the distorting action of a different *SD* chromosome from that which engendered the change in the X so that the specificity of the induced modifier is not restricted to the particular *SD* that generated it. It may be noted that this experiment (and the previous one with SD^{in}) is further evidence that *SD* itself does not have to pass through a female in order that conditional distortion occur.

The experiments with SD^{in} given above indicate that a male does not actually have to exhibit segregation-distortion in order to induce an X chromosome. This is so for the reason that the $SD^{in}/cn\ bw$ males used in that experiment show normal, 1:1, ratios (SANDLER and HIRAIZUMI 1960a). A further check on this point made use of the fact that *SD/In(2LR)Cy* males show no distortion (SANDLER, HIRAIZUMI and SANDLER 1959). In this case, *R(SD)-4/In(2LR)Cy* males

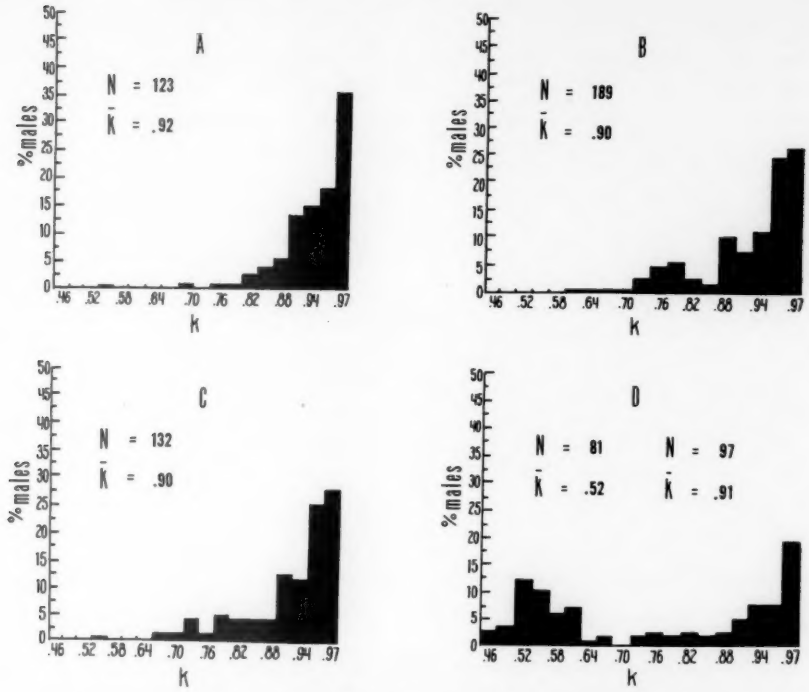

FIGURE 5.—A demonstration of the changed state of the X chromosome. Histogram A = the sons of *R(SD)-4* males crossed to compound X and Y-bearing females selected to be unconditioned; B = the *R(SD)-4* sons of these same males crossed to *SD-5/cn bw*, free X females; C = the same as in "A"; D = the *R(SD)-4* sons of the same males as in "C" crossed to *SD-5/cn bw*, free X females.

were crossed to *cn bw/In(2LR)Cy* females, and the F_1 *R(SD)-4/In(2LR)Cy* female progeny were tested to see whether any were conditioned. Conditioned females were found in this case, confirming the conclusion that an *SD* male can induce the X chromosome whether or not he actually exhibits segregation-distortion.

A final question about conditional distortion to be considered is the fate of an induced X chromosome in the absence of *SD*. To determine this, *R(SD)-4/cn bw* males were mated to *cn bw/In(2LR)Cy* females, and the F_1 *R(SD)-4/In(2LR)-Cy* females were crossed individually to *cn bw/cn bw* males. From this cross, *R(SD)-4/cn bw* males were tested to determine whether the female was conditioned or not, and, from the conditioned females in this same cross, *cn bw/In(2LR)Cy* males (two or three per female) were crossed to *y f*:=/Y; *R(SD)-4/In(2LR)Cy* females. From this cross, *R(SD)-4/cn bw* males were tested for segregation-distortion. The control consisted of testing similar males from the

TABLE 1

The behavior of induced and not induced X chromosomes after one generation in males without SD. *The origin of the males in each set in the table is given in the text*

k	Set I	Set II	Set III
0.43	...	...	14.63
0.46	...	...	9.76
0.49	...	...	26.83
0.52	...	...	17.07
0.55	...	...	14.63
0.58	...	...	12.20
0.61	...	...	2.44
0.64	...	...	0.00
0.67	...	...	2.44
0.70	...	...	...
0.73	...	2.08	...
0.76	...	2.08	...
0.79	1.16	2.08	...
0.82	0.00	0.00	...
0.85	2.33	2.08	...
0.88	1.16	2.08	...
0.91	3.49	0.00	...
0.94	3.49	2.08	...
0.97	88.37	87.50	...
N	86	48	41
$\bar{k}$	0.99	0.98	0.52

unconditioned grandmothers. Pedigrees were kept. The results are given in Table 1.

From each of four unconditioned *R(SD)-4/In(2LR)Cy* females, two or three *cn bw/In(2LR)Cy* males were collected and crossed to the compound X *SD* females. These yielded *R(SD)-4/cn bw* test males—the k value distribution of which is given in the first column (Set I) of the table. It can be seen that this is the standard semistable distribution indicating that the X chromosome from unconditioned females is unchanged. From four conditioned females, ten *cn bw/In(2LR)Cy* males were also collected (again two or three per female) and their *R(SD)-4/cn bw* sons tested for k. Here, two different kinds of sibships were observed. From five of the males, the sibships produced the usual semistable distribution. The k value distribution from these males is given in the second column (Set II) of the table. This is undoubtedly the distribution from the unchanged (i.e. grandmaternal) X chromosome from a conditioned female and indicates that nothing happens to that X in such females. Finally, the other five *Cy* sons produced a distinctly different kind of *R(SD)-4* male sibship—the k value distribution of which is given in the last column (Set III) of Table 1. This almost certainly corresponds to the behavior of the induced X after one generation in males lacking *SD*.

From these results it is clear that, for one generation at least, the induced modifier on the X chromosome remains unchanged. This is shown both by the observation that one half (five out of ten) of the sons of conditioned females show low k distributions among their progeny, and also by the fact that the low k distributions themselves vary uniformly about a k value equivalent to that characteristic of the changed X in the first generation.

Evidence for "conditioning states" of SD: The *R(SD)-4* stocks used throughout the studies reported here were kept by selecting, in every generation, one or two single *R(SD)-4/cn bw* males and backcrossing these to *cn bw* females. We have observed, during the two years that these studies were carried out, at least three (and possibly more) distinct kinds of results: (1) for some generations at a time, the *R(SD)-4* males would produce only unconditioned daughters; (2) for some generations, only conditioned daughters are observed; (3) most often, about 15–30 percent of the females are conditioned. From these observations, it seems reasonable to suppose that there are at least three different kinds of *SD* males—each defined by the proportion of induced X's that it transmits to its progeny.

Additional evidence on this point is provided by the results of crossing *R(SD)-4* males to compound X females. Here, two kinds of results have been observed: either all the sons of a single male crossed with compound X females exhibit no distortion (Figure 3; histogram D) or else a small fraction do (Figure 3, histogram B). The former presumably correspond to males that produce only conditioned daughters (although this conclusion was not checked directly). The latter result requires consideration. A comparison of histograms A and B in Figure 3 reveals that about 20 percent of the males from the compound X female cross exhibit lower k values than the corresponding free X cross (in spite of the fact that both sets of males result from precisely the same fathers by double mating). It was considered possible that males that produce sons showing the distribution of histogram B, Figure 3, would produce the typical 15–30 percent conditioned females. Accordingly, the daughters of males whose sons (from a mating to $y\ f/=$ females) showed a distribution similar to histogram B, Figure 3, were tested. It was indeed found that of 37 females tested, seven were conditioned. Thus, the two kinds of results given in Figure 3 illustrate two of the possible "conditioning states" of *SD*. The state giving no induced X's was not observed in this experiment.

Although the proportions of low k values agree between the compound X and free X (through female) crosses, the k values themselves do not. A conditioned female produces 50 percent sons with normal 1:1 segregation ratios, while the lower k values in histogram B, Figure 3, range from 0.50 to more than 0.85. This matter requires further study, but it seems likely that the induced X is somehow stabilized in a female. The entire question of the fate of the induced X in females and the matter of conditioning states is under additional study.

On the differences among different SD *lines:* Until this point, all of the experiments have utilized the *SD*-bearing recombinant chromosome, *R(SD)-4*. This chromosome is typical of recombinants between the original *SD* line, *SD-72*, and

cn bw but differs markedly from other types of *SD* lines in its response to the specifically modified X chromosome.

There were two different types of *SD* lines originally collected in nature—*SD-5* (that contains two inversions and a lethal in IIR) and *SD-72* (that contains one inversion and no lethal). It has already been shown that *SD-5* (and thus presumably all *SD* lines) regularly generate changed X chromosomes in the same way as *R(SD)-4* (see Figure 5 and accompanying text). The effect of such changed X chromosomes on the behavior of *SD-5* and *SD-72*, however, is very much less marked than it is on *R(SD)-4*. Thus Figure 6 shows the results of

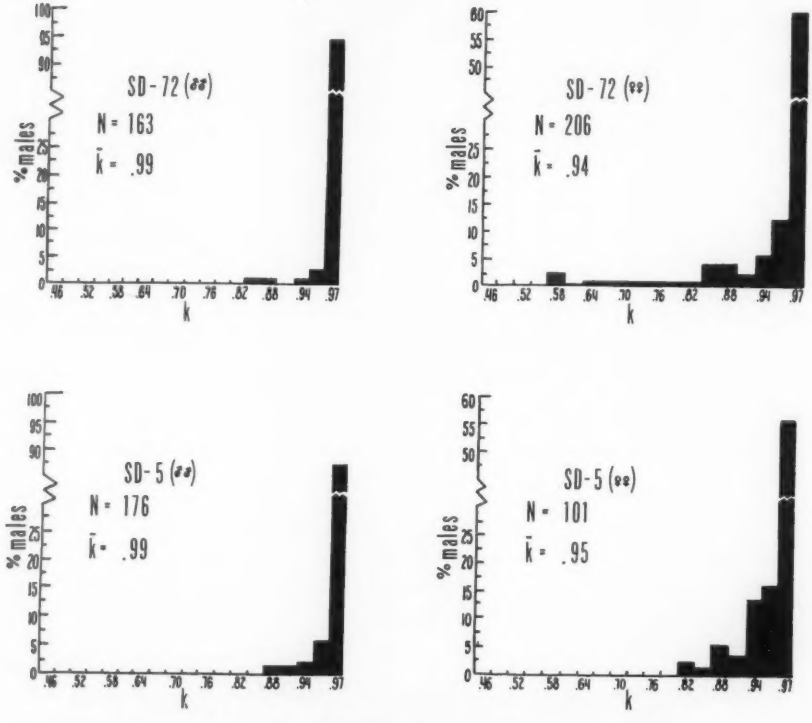

FIGURE 6.—The results of passing *SD-72* and *SD-5* through males and females.

passing *SD-5* and *SD-72* through females as compared with males. It is clear that whereas there is a slight lowering of the *k* values in males that receive their *SD*-bearing chromosome from the female parent, there is no evidence for the complete suppression of segregation-distortion such as is observed in the case of *R(SD)-4*.

The typical recombinant between *SD-5* and *cn bw* results from an exchange

distal to the inversions and the lethal so that the *SD*-bearing chromosome, symbolized *R(SD-5)* followed by an identifying number, carries both inversions and the lethal. Recombination between *SD-72* and *cn bw*, on the other hand, takes place proximal to the inversion so that these *SD*-bearing recombinants, symbolized *R(SD)* followed by an identifying number, contain no gross rearrangements. In the case of either type of recombinant, the *k* value distribution will vary somewhat from male to male (in which case the line is referred to as "semistable") or the male to male variation may be very wide (the line then being termed "unstable"). The semistable and the unstable states differ from the original, "stable", state in that the recombinant *SD* chromosome has lost by crossing over a stabilizing modifier, *St(SD)*, located at the tip of IIR. A semistable line can become unstable without further recombination. The evidence bearing on the matter of stability has been presented by SANDLER and HIRAIZUMI (1960).

Samples of the various kinds of recombinants have been tested for their response to changed X chromosomes by comparing the distribution of *k* values when the *SD*-bearing chromosome has been inherited from females as compared with males. The results are given in Table 2. It can be seen that in the case of

TABLE 2

The distribution of k *values of recombinant* SD*-bearing chromosomes inherited from the male, as compared with the female, parent. The entries are the proportion of males falling into each* k *value interval*

	SD-5 Recombinants						*SD-72* Recombinants					
	R(SD-36)-1*		R(SD-5)-22		R(SD-5)-18		R(SD)-8		R(SD)-2		R(SD)-5	
k	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀†	♂♂	♀♀†
0.43	...	0.86	...	...	...	...	...	7.50	...	6.45	...	13.04
0.46	...	3.42	...	...	...	...	1.12	5.00	0.97	4.84	...	0.00
0.49	1.54	2.56	...	0.83	...	...	0.00	10.00	0.00	9.68	...	21.74
0.52	6.15	2.56	...	0.83	...	...	0.00	5.00	0.00	14.52	...	0.00
0.55	3.08	11.11	...	0.00	...	...	3.37	7.50	0.00	6.45	...	17.39
0.58	3.08	5.13	1.45	0.00	...	...	4.49	0.00	0.00	11.29	...	0.00
0.61	3.08	3.42	0.00	0.00	...	...	3.37	0.00	0.00	1.61	...	4.35
0.64	4.62	2.56	0.00	0.83	...	0.81	5.62	2.50	0.00	6.45	...	0.00
0.67	1.54	6.84	0.72	1.65	...	0.00	4.49	5.00	0.97	0.00		0.00
0.70	6.15	2.56	1.45	3.31	..	0.00	11.24	0.00	4.85	0.00	...	0.00
0.73	4.62	3.42	0.72	4.96	0.78	0.00	4.49	2.50	2.91	0.00	...	0.00
0.76	7.69	2.56	0.72	4.96	0.00	0.00	3.37	2.50	1.94	0.00	...	0.00
0.79	6.15	4.27	0.72	7.44	0.00	0.81	0.00	0.00	6.80	0.00	...	0.00
0.82	4.62	0.00	1.45	12.40	0.78	0.81	7.89	5.00	8.74	1.61	...	0.00
0.85	6.15	1.71	5.07	9.92	1.55	0.81	6.74	2.50	11.65	0.00	1.85	0.00
0.88	3.08	1.71	5.80	7.44	6.98	1.63	11.24	0.00	19.42	0.00	8.33	0.00
0.91	10.77	5.98	11.59	17.36	3.88	8.94	16.85	7.50	15.53	3.23	9.26	0.00
0.94	6.15	8.55	28.26	9.09	18.60	13.82	5.62	2.50	9.71	3.23	20.37	0.00
0.97	21.54	30.77	42.03	19.01	67.44	72.36	10.11	35.00	16.50	30.65	60.18	43.48
Total ♂♂	65	117	138	121	129	123	89	40	103	62	108	23
$\bar{k}$	0.81	0.79	0.94	0.91	0.97	0.90	0.81	0.77	0.88	...	0.95	...

* *SD-36* is the same type as *SD-5*.
† Conditioned females only were chosen for this line.

recombinants from *SD-5* type lines the response to the changed X chromosome is similar to that of the original *SD-5* line, while in the case of *SD-72* recombinants, such as *R(SD)-4*, the response is (1) extreme and (2) independent of whether the recombinant is semistable or unstable.

Two tests were performed to determine whether some of the nonreactivity of the original *SD* lines to the changed X was the result of the presence of *St(SD)* on the stable *SD*-bearing chromosomes. Thus *R(SD)-4/In(2LR)Cy* conditioned females (selected on the basis of the k value distribution of their sons) were crossed to *cn bw/St(SD)* males and the *R(SD)-4/cn bw* and *R(SD)-4/St(SD)* sons were tested for distortion. The origin of *St(SD)*-bearing chromosomes can be found in SANDLER and HIRAIZUMI (1960). These results are presented in Table 3. It is clear that whereas the control mating, *R(SD)-4/cn bw*, shows the normal conditioned behavior the experimental set, *R(SD)-4/St(SD)*, shows no complete supression of distortion. Similarly, a *R(SD)-4* chromosome, onto which *St(SD)* has been placed by crossing over, was made heterozygous with *In(2LR)Cy* in females and these females crossed to *cn bw* males. The resultant *R(SD)-4, St(SD)/cn bw* males were tested for distortion. The results are shown in Figure 7. Here again there is no evidence for completely suppressed distortion. It is clear, therefore, that *St(SD)* causes a nonreactivity of *SD* to the specifically modified X chromosome.

This observation satisfactorily accounts for the results from *SD-5*, *SD-72* and

TABLE 3

Conditioned R(SD)-4/In(2LR)Cy *females were crossed to males heterozygous for* St(SD) *and* cn bw. *The* F_1 R(SD)-4/St(SD) *and* R(SD)-4/cn bw *males were collected and tested for distortion*

k	*R(SD)-4/cn bw*		*R(SD)-4/St(SD)*	
0.43	3.23			
0.46	3.23			
0.49	12.90	N = 20		
0.52	16.13			
0.55	12.90	$\bar{k} = 0.54$		
0.58	6.45			
0.61	9.68			
0.64				
0.67	3.23			
0.70	0.00			
0.73	3.23		3.57	
0.76	0.00		0.00	
0.79	0.00	N = 11	3.57	
0.82	0.00		0.00	N = 28
0.85	0.00	$\bar{k} = 0.92$	14.29	
0.88	0.00		0.00	$\bar{k} = 0.94$
0.91	6.45		14.29	
0.94	0.00		10.71	
0.97	22.58		53.57	

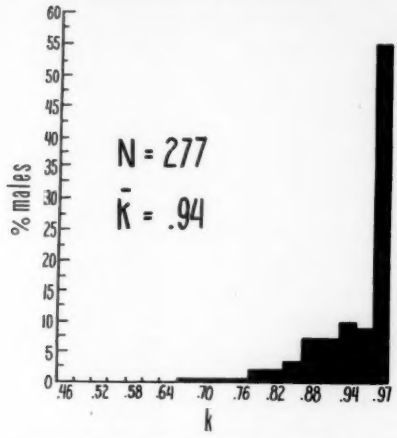

FIGURE 7.—The response of *R(SD)-4* carrying the stabilizing modifier, *St(SD)*, to the specifically modified X chromosome.

the recombinants from *SD-72*. It fails to explain, however, the nonreactivity of recombinants from *SD-5*, since these too lack *St(SD)* but are nevertheless nonreactive. Accordingly it must be supposed that either (1) *SD-5* carries a nonreactive allele of *SD* or (2) there exists, on *SD-5*, an additional nonreactivity modifier proximal to *St(SD)*. This question has been resolved by testing a rare type of recombinant from *SD-5*, *R(SD-5)-32*. This recombinant carries neither the inversions nor the lethal indicating that the recombination between *SD-5* and *cn bw* took place between *cn* and the proximal break point of the proximal inversion. The recombinant line is unstable.

Conditional distortion, in the case of *R(SD-5)-32*, was tested by doubly mating *R(SD-5)-32/cn bw* males to homozygous *cn bw* free X and *y f*:=/Y; *cn bw*/*cn bw* females and testing the F_1 *R(SD-5)-32/cn bw* males for distortion. These results are presented in Table 4. It can be seen that the males resulting from the compound X cross yield either a distribution of *k* values not different from the free X cross or all nondistorting sons as in the compound X crosses recorded above for *R(SD)-4* Consequently, it is evident that *R(SD-5)-32* does react to the changed X in the same way as *R(SD)-4*, and therefore *SD-5* does not contain a nonreactive allele of *SD*. Accordingly *SD-5* must be supposed to carry an additional, more proximally placed, modifier of conditional segregation-distortion. Whether this is a simple modifier or some action of the inversions themselves is not known.

DISCUSSION

It is clear that *SD* has the special property of inducing a specific inhibitor of its own action on the X chromosome. From the arguments presented in this report, particularly the fact that the very males that transmit the specifically

modified X chromosome themselves began life with an unchanged X, it is evident that this is specific induction as opposed to segregating modifiers in the *cn bw* stock. It is possible, however, that X chromosomes could be found that are not inducible in this way. If this is true, then it is conceivable that the other chromosomes in the *cn bw* stock (that is the Y chromosome and chromosomes III and IV) are simply not inducible but that stocks could be found in which chromosomes other than the X would be specifically modified by *SD*. That such inductions do not occur in the *cn bw* background follows immediately from the fact that males showing normal segregation ratios occur only as sons of *SD* females and not as sons of *SD* males (if it is supposed that the changed property is dominant; if, on the other hand, the induced property is recessive, then there could be inductions of other chromosomes that would not be detected in these experiments). In general, then, there is no reason from this analysis to conclude that *SD* and some section of the X chromosome are particularly related. The relationship exhibited here may simply be a consequence of the specific genetic background and experimental design that happens to have been utilized.

It is interesting to inquire just when, in an *SD* male, the X chromosome becomes changed. The answer to this question at this time is not at all clear. At first sight it would seem as though the change must occur after meiosis, for, should it have been completed before meiosis, the male in which it occurred would show

TABLE 4

The sons of R(SD-5)-32 *males crossed to (A) free X females, (B) compound X and Y-bearing females and yielding an unconditioned distribution, and (C) compound X and Y-bearing females and yielding a conditioned distribution*

k	A	B	C
0.43	...	...	8.21
0.46	...	...	13.43
0.49	...	...	12.69
0.52	...	6.90	22.39
0.55	1.52	0.00	17.91
0.58	6.06	6.90	12.69
0.61	3.03	13.79	8.21
0.64	7.58	0.00	1.49
0.67	3.03	3.45	2.24
0.70	7.58	10.34	0.75
0.73	3.03	3.45	...
0.76	6.06	3.45	...
0.79	7.58	10.34	...
0.82	7.58	6.90	...
0.85	7.58	0.00	...
0.88	10.61	0.00	...
0.91	12.12	10.34	...
0.94	10.61	6.90	...
0.97	6.06	17.24	...
Total ♂♂	66	29	134
$\bar{k}$	0.81	0.78	0.54

a normal segregation ratio. On the other hand, if the change in the X chromosome occurs either during or after meiosis, then it is not immediately evident why all, or almost all, of the X's from a given male are, in some instances at least, specifically modified. It seems that there is no way of deciding between these alternatives and that all that can be said is that either the X chromosome becomes changed early in ontogeny but for some reason the phenotype does not manifest itself until the succeeding generation or that the change takes place during or after meiosis and that the inducing mechanism may, nevertheless, work in every germ cell.

The stabilizing modifier, *St(SD)*, apparently has two distinct functions. On the one hand it stabilizes the action of *SD* at a high level of activity (SANDLER and HIRAIZUMI 1960), and, on the other, it imparts a high degree of nonreactivity of *SD* to the effect of the specifically modified X chromosome. It might initially be imagined that these are two manifestations of the same activity of *St(SD)* which is simply to enhance the distorting action of *SD* under all circumstances. The true situation, however, while possibly this simple, is probably somewhat more complex. It has been shown above that in addition to *St(SD)* there is another modifier on *SD-5* affecting the response of *SD* to the changed X. This additional modifier does not affect stability since *R(SD-36)-1*, for example, is extremely unstable but nevertheless does not react to the changed X (see Table 2). Thus, irrespective of the nature of *St(SD)*, specific modification of the response of *SD* to the changed X chromosome does occur (this is specific modification of specific modification!). It seems most reasonable, therefore, that *St(SD)* also specifically modifies the response of *SD* to the changed X, and therefore, that *St(SD)* does indeed have two functionally distinct actions. It should be mentioned however, that there is no way in these experiments to distinguish between two functions of *St(SD) versus* the possibility of another modifier closely linked to *St(SD)* having the second function.

It is immediately evident that the equality of distorting and nondistorting sons of conditioned females implies that the specific modifier induced on the X chromosome by *SD* is a single locus. This is so for the reason that if more than one locus were either necessary or sufficient to inhibit distortion, the segregation into two classes would depart from equality. It would be interesting therefore to determine the location of this induced modifier. However, a number of marked X chromosomes isolated for this purpose all contained modifiers of distortion (as does the Muller-5 chromosome) which made them useless for this purpose. When, moreover, attempts were made to separate the marker from the modifier, the results were difficult to interpret, for it was uncertain that a marked chromosome lacking modifiers had been produced. For this reason, it was considered reasonable to defer mapping the induced modifier until the location and behavior of the already existing modifiers is worked out.

From the standpoint of the evolution of the phenomenon of segregation-distortion, conditional distortion is of interest in two connections. In the first place, it would seem that the ability of *SD* to cause a specific change on the X is, from

the standpoint of *SD* itself, selectively disadvantageous. Thus this is very unlikely to have evolved in response to selection and therefore is very likely to be an integral part of *SD*. That is, it seems reasonable that potential distorting ability implies the ability to induce the X chromosomes. The fact that the *SD* subelement itself, can induce the X also supports this notion. It is, however, immediately evident why the modifiers of the phenomenon of conditional segregation-distortion should have persisted. Given their action, the ability of *SD* to change the X chromosome into a specific inhibitor of its own action becomes relatively unimportant because in nature all of the chromosomes carry the modifiers of *SD*'s response to the specifically modified X chromosome.

In this general connection an additional point may be noted. It has been found that stocks either from the laboratory or those collected in nature contain numerous modifiers of segregation-distortion (SANDLER and HIRAIZUMI 1959 and unpublished). These modifiers probably may be located on any of the chromosomes of the complement. It is not unreasonable that these modifiers are present in populations as a consequence of selection against distortion during a prior history with *SD*. Indeed, the fact that *SD* has had a long evolutionary history has been argued by SANDLER and HIRAIZUMI (1959, 1960), HIRAIZUMI, SANDLER and CROW (1960) and most cogently by MANGE (1961), who reported on independent collection of *SD* from a natural population in Lower California. It is probable, therefore, that modifiers of segregation-distortion arise in natural populations and are caused to increase in frequency by virtue of natural selection (HIRAIZUMI, SANDLER and CROW 1960). It also may be that these modifiers arise specifically in response to the *SD* element. It is not impossible that the specific modification induced on the X chromosome (or, indeed, on any chromosome) could, in some cases, prove permanent or it could be that specific modification repeated over a number of generations, as would happen in natural populations in which *SD* was fairly common, would finally result in permanent modifiers of the sort observed now. Evidently, a matter such as this cannot be rigorously resolved (particularly, since the modifiers observed have properties rather different from those of the induced X, the observed heritability of the induced modifier on the X is not immediately relevant), but it is worth noting that the phenomenon of conditional segregation-distortion opens the possibility that *SD* engenders its own modifiers.

It may be noted that the conditional distortion system itself and the intricacy of *SD*'s response to this phenomenon (that is the development of specific modifiers) suggests a long evolutionary history of segregation-distortion.

It is interesting to note that regular, heritable, specific inductions of alleles are observed in maize in the case of the phenomenon of paramutation (BRINK 1958), the apparently similar changes at the *B* locus (COE 1959) and the well-known modifier of a_1, *Dt* (RHOADES 1938). Although there is a definite similarity between these cases in maize and the phenomenon of conditional distortion, it is not yet clear just how—if at all—these various phenomena are to be analogized.

Finally, the phenomenon of conditional segregation-distortion is, most prob-

ably, a case of "nonallelic conversion"; that is, an instance of a heritable, directed, gene change that cannot readily be imagined to come about by any kind of copy-choice mechanism.

SUMMARY

In males carrying the *Segregation-distorter* element *(SD)* the X chromosome may become changed into a specific inhibitor of segregation-distortion. It has been shown that if *SD* males are crossed to normal free X females and the *SD*-carrying female progeny crossed to normal males, then among the heterozygous *SD* grandsons only one half exhibit segregation-distortion; the other half, those receiving their X chromosome from the *SD*-carrying maternal grandfather, show normal 1:1 segregation ratios. In the next generation, however, all of the males receive their X chromosome from normal females, and hence all exhibit segregation-distortion. Using marked X chromosomes, it is possible to show directly that the chromosome responsible for the suppression of distortion is the X chromosome from the maternal grandfather.

That this is specific induction is shown first by the fact that the male that produces either sons that do not distort (if the male is crossed to compound X females) or daughters that produce sons, half of which do not distort (if the male was crossed to free X females), himself exhibits the phenomenon of segregation-distortion. Secondly since the *SD* line is kept by backcrossing heterozygous *SD* males to the same standard tester stock generation after generation, the X chromosomes received by a male from his mother are a sample of precisely the same X chromosomes that are delivered by a heterozygous *SD* female when such a cross is made. Thus if this were not specific induction of a modifier on the X chromosome, it would be expected that males that do not distort would be produced as frequently as the sons of *SD* males as of the sons of *SD* females which is not the case.

This phenomenon of induction on the X chromosome occurs apparently in all *SD* lines. However, the response of *SD* to the specifically modified X differs from line to line. In particular, it has been found that there are modifiers that cause *SD* to be relatively nonreactive to the specifically modified X. One such modifier is the stabilizer of *SD*, *St(SD)*, that has been previously shown to reduce male to male variability in segregation ratios in *SD* lines.

The exact nature of the conditioned state is not understood. It is known that the state is defined by the X chromosome having been changed in an *SD* male. Apparently, *SD* males will transmit either all induced X's or a small fraction of induced X's or, possibly, no induced X's. How such males differ from one another is not at present understood.

Various other properties of the phenomenon of conditional segregation-distortion and some of the evolutionary possibilities of this phenomenon are discussed.

Finally, the phenomenon of conditional segregation-distortion exhibits two unique properties: (1) that the *SD* element induces a change on the X chromo-

some which in turn modifies the action of *SD*; and (2) this specific induction appears to be stable and comes about presumably without physical contact between *SD* which is on the second chromosome and the induced site on the X so that the usual copy-choice interpretation of conversion phenomena seems rather unlikely.

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GENE DOSAGE AND ENZYME ACTIVITIES IN DROSOPHILA MELANOGASTER[1]

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THE relative ease with which the Drosophila genome can be manipulated made this fly the subject of early investigations on gene dosage. Morphological expression was found to be sensitive to the relative numbers of the different chromosomes and to additions or subtractions of single loci or chromosome fragments. STERN (1929) found that increased gene dosage could give greater type effect, and MULLER (1932) showed that less or even new type effect could be similarly obtained.

BRIDGES (1939) found that sex is determined in Drosophila by interactions between X chromosomes and autosome sets. Later studies with aneuploid flies have shown that contributions toward femaleness or maleness are made by numerous genes scattered on the X chromosomes and autosomes, respectively. This evidence has recently been reviewed by PIPKIN (1959).

Studies of Drosophila triploids, intersexes and superfemales have shown that chromosomal interactions may complicate the effects of artificially produced gene dosage changes. For example, STERN (1929) found that three X chromosomes bearing the bristle mutant "bobbed", gave a phenotype closer to the normal in superfemales than in triploid females. SCHULTZ (1935) found that triploid females required more fourth chromosomes containing the bristle mutant "shaven", to approach the wild type, than were required by diploid females. Such interactions can be further compounded by the background of mechanisms (MULLER 1932) that have evolved to compensate for the dosage changes related to the X chromosome difference between sexes.

Physiological and biochemical differences as well as morphological ones have been reported to follow changes in gene dosage. Investigations of fertility, viability and related characteristics have indicated that all degrees of aneuploidy are, in general, detrimental to animals and plants (LINDSTROM 1936; GOODSPEED and AVERY 1939; BURDETTE 1940; PATTERSON, BROWN and STONE 1940). FOX (1959) found two antigenic differences between male and female Drosophila that were independent of the Y chromosome and one that was correlated with its presence. So far, the biochemical mechanisms responsible for any of the dosage induced phenotypic changes remain obscure.

The present study was made to investigate the effects of changes in gene

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dosage on enzymatic activities in homogenates from *Drosophila melanogaster*. Five enzymes (aconitase, guanase, alcohol dehydrogenase, alkaline phosphatase and fumarase) were chosen for which relatively precise assays were available and which were apparently active in Drosophila homogenates. These activities were then compared among diploid males and females, superfemales and triploid females.

MATERIALS AND METHODS

Flies were raised at 22–26°C in uncrowded half-pint bottles containing yeasted corn meal or banana medium. The parents of the flies to be tested were transferred daily to fresh food. Usually, a sufficient number of flies for testing could be obtained from a single day's egg-laying. Superfemales were delayed in development, however, and were compared to sibs taken from later cultures from their transferred parents.

Equal numbers of each genotype to be tested were harvested within three hours after emergence and then starved for 12 to 24 hours on paper moistened with water. The flies were then ground alive in distilled water (150–300 flies in three ml) in a Ten Broeck glass homogenizer. The grinding and all subsequent handling of the homogenate was performed at 0–5°C. The homogenate was extracted further by a 15 to 30-minute wait and then centrifuged for 30 minutes at 80,000 $\times g$. The supernatant was drawn from beneath a fatty layer and used, as soon as possible, as the source of the enzyme activities. Before assaying, the supernatants to be compared were diluted to identical protein concentrations. Protein was measured by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951).

Enzyme activities were measured as optical density changes at the wave lengths listed in Table 1. The assays were run in 1 ml quartz cuvettes, at 30°C. within a model DU Beckman spectrophotometer that was equipped with a model 5800 Beckman energy-recording adaptor and Brown automatic recorder. Optical density changes were recorded through the first two minutes of each reaction during which time the reaction rates were linear in all of the assays, at all of the protein concentrations used. All of the reaction rates were also directly proportional to the concentration of protein used in the assays (Figures 1 and 2).

A similar protocol was observed for all five assays. Buffer, homogenate and

TABLE 1

*Enzyme activity assay components**

Activity	Buffer (0.15M)	Substrate μmoles/ml	Wave length mμ
Aconitase	Tris-HCl, *p*H 7.0	D-isocitrate, 20	240
Guanase	K phosphate, *p*H 5.8	guanine, 0.1	245
Alcohol dehydrogenase	glycine NaOH, *p*H 9.5	ethanol, 20 DPN (0.1 mg/ml)	340
Alkaline phosphatase	Tris-HCl, *p*H 8.5	*p*-nitrophenyl-phosphate, 8	410
Fumarase	K phosphate, *p*H 7.0	fumarate, 25	300

* The listed values are the initial concentrations of the reaction-mixture components.

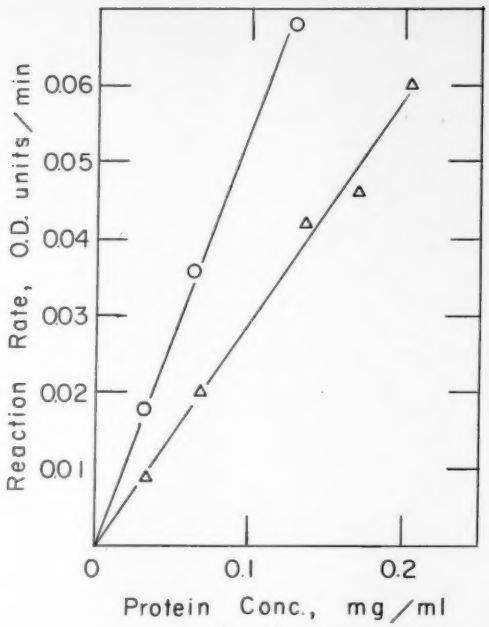

FIGURE 1.—The variation of reaction rate with protein concentration. Circles denote values for aconitase activity, triangles for alcohol dehydrogenase activity.

distilled water (as well as ethanol for the alcohol dehydrogenase assay) were brought to the respective temperature during a ten minute incubation. The volumes used were such that the addition of substrate, to initiate the reaction, brought the reaction mixture volume to one ml and the concentrations of buffer and substrates to those shown in Table 1. Control blanks without substrates were run for each assay and were negative for the protein concentrations dealt with in this report.

The buffers and the *p*H values chosen for each assay were those that gave maximum measured rates. The *p*H optimum for *p*-nitrophenyl phosphate degradation is on the acid side (below *p*H 5.5), but the alkaline *p*H was chosen in order to follow *p*-nitrophenol generation spectrophotometrically.

The assays reported here are all modifications of procedures previously reported by workers with other organisms. The basic assays are listed under the names of the enzymes in volumes I and II of the series edited by COLOWICK and KAPLAN (1955). Aconitase and fumarase activities were previously reported in Drosophila homogenates by SPIRTES (1951) and ZIEGLER-GÜNDER and JAENICKE (1959). Phosphatase activity has been detected in Drosophila tissue sections through cytochemical stains (KRUGELIS 1946).

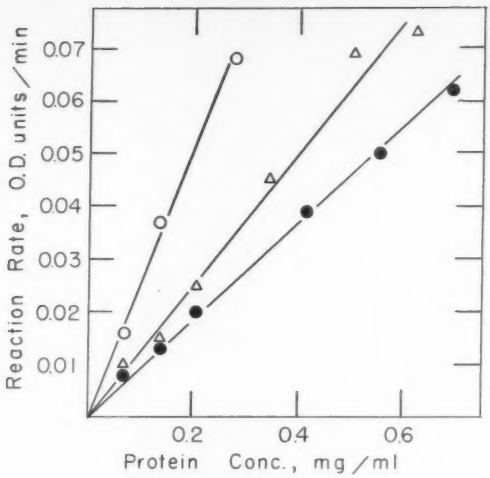

FIGURE 2.—The variation of reaction rate with protein concentration. Open circles denote values for alkaline phosphatase activity, triangles for guanase activity, shaded circles for fumarase activity.

Guanine hydrochloride and potassium fumarate were obtained from the Nutritional Biochemicals Corporation. Sodium isocitrate (dl + allo), *p*-nitrophenyl phosphate, *p*-nitrophenol, *cis*-aconitic anhydride, DPN and bis(*p*-nitrophenyl) phosphate were obtained from the Sigma Chemical Company.

The symbols used below to identify mutations are as follows: yellow (y, y^1, y^2, y^{31d}); vermilion (v); forked (f); scute (sc); white apricot (w^a); echinus (ec); scute^8 (sc^8); diminutive (dm); Bar (B); cinnabar (cn); inversion (1) EN [$In(1)EN$]; transformer (tra); short arm of the Y chromosome (Y^S); long arm of the Y (Y^L).

RESULTS

For each experiment the flies of the two compared genotypes were reared in mixed culture, processed in parallel, and triplicate measurements were made of the five activities in each homogenate. The experiments were performed over a period of several months, during which time certain sources of between-experiment variability (such as larval nutrition, protein determination, time between grinding and assaying) could not be strictly controlled. Therefore, no attempt was made to establish typical values for Drosophila enzyme activities. Only the differential responses between genotypes within an experiment, and not the absolute activity levels, were used to compare the genotypes.

Appropriate tests showed that the guanase, alcohol dehydrogenase and alkaline phosphatase activities were quite stable. No changes were observed during a five-

hour period (at 0–5°C) after obtaining the supernatant. The fumarase activity dropped about five percent during this time and the aconitase about 30 percent. The errors due to enzyme instability during each comparison were rendered negligible by staggering the triplicates. In order to convey a measure of the true rates of the activities, the values in Table 4 have been expressed in terms of substrate turnover (assuming all of the optical density changes were due to the listed enzyme).

Diploid males vs. *diploid females*: The measurements of the five activities in males and females were repeated in six separate experiments. Four of the six were made with homogenates from flies of wild-type strain Oregon-R. The fifth was a comparison of progeny from the cross of males from a wild-type strain recently collected near Austin, Texas and females of genotype $\underline{y\ v\ f}$/Y from a Texas laboratory stock (Cross 5). The sixth was a comparison between progeny from Oregon-R males and females of genotype $\underline{y^1}$/Y received recently from the Oak Ridge National Laboratory (Cross 6).

Table 2 gives typical rates for the triplicate determinations in one experiment. Because the precision within triplicates was very good, only the means were used for calculations. The ratios of male to female activities were similar in all six experiments and were averaged to give the means and limits listed in Table 2.

These six pooled comparisons represent all that were made with the experimental technique given above; no data were discarded. Earlier preliminary experiments had been done with flies that had been frozen overnight before grinding and the results reported elsewhere (SEECOF 1960). This technique, however, was found to give more variable and therefore misleading results.

TABLE 2

Enzyme activity comparisons between diploid males and females

	Aconitase	Guanase	Alcohol dehydrogenase	Alkaline phosphatase	Fumarase
Male*	0.677	0.153	0.340	0.159	0.073
	0.693	0.149	0.386	0.174	0.070
	0.677	0.165	0.371	0.174	0.068
	0.682	0.156	0.366	0.169	0.070
Female*	0.793	0.161	0.363	0.189	0.108
	0.776	0.157	0.416	0.197	0.114
	0.743	0.153	0.371	0.189	0.110
	0.777	0.157	0.383	0.192	0.111
Male/female†	0.878	0.994	0.956	0.880	0.631
Male/female‡	0.87	0.98	1.01	0.93	0.60
S.E.	0.080	0.010	0.030	0.029	0.063

* The values listed are rates (ΔO.D./min/mg protein) obtained in a typical experiment (triplicate determinations and averages).
† The male/female ratio of activities in this experiment.
‡ The means and standard errors of the male/female ratios from six pooled experiments.

TABLE 3

*Enzyme activity comparisons among various genotypes**

	Aconitase	Guanase	Alcohol dehydrogenase	Alkaline phosphatase	Fumarase
S♀†	0.748	0.125	0.269	0.204	0.114
♀†	0.882	0.135	0.278	0.215	0.128
S♀‡	0.492	0.149	0.353	0.276	0.092
♀‡	0.460	0.155	0.353	0.267	0.070
♀ (3N)	0.660	0.110	0.250	0.135	0.048
♀ (2N)	0.695	0.120	0.240	0.135	0.050
♂ —Y	0.775	0.197	0.460	0.324	0.119
♂ +Y	0.690	0.207	0.517	0.345	0.103

* The listed values are rates expressed as ΔO.D./min/mg protein. Each rate is the average of a determination made in triplicate. Only the two rates within each pair are directly comparable.
† Cross 5. S♀ means superfemales.
‡ Cross 6.

Superfemales vs. *diploid females*: Superfemales were obtained from Crosses 5 and 6. The activities were compared once for each cross. The differences recorded between females and superfemales were considered to be nonsignificant (Table 3).

Triploid females vs. *diploid females*: Triploids and diploids were compared once. Lack of sufficient homogenate from triploids prevented the measurement of all five activities in a single experiment. The aconitase and fumarase activity readings came from triploids of the genetic constitution *y² sc u^m ec*/FM4 *y^+1d sc^s dm B; cn/cn/cn* and diploid females which were *y² sc u^m ec; cn/cn*. The guanase, alcohol dehydrogenase and alkaline phosphatase activity readings were obtained from triploids which were *v f/ y B* and diploid females which were *v f*. No significant differences were obtained (Table 3).

Males with Y chromosomes vs. *males without Y chromosomes*: Males without Y chromosomes came from the cross of Oregon-R females to Y^s $X{\cdot}Y^L$ (Y^s *In(1)EN, B y*·Y^L) males. Males with Y chromosomes came from the cross of *y/y* females to Oregon-R males. The two kinds of males were reared together but, unlike the flies in the other experiments reported here, they were kept on two percent sucrose for the 12 to 24 hours prior to grinding. However, no obvious differences in enzyme activities were recorded (Table 3).

Males and females raised under sterile conditions: The sterile flies (strain Oregon-R) were harvested from an old culture and were therefore of mixed ages. They were processed as detailed above except that they were ground immediately after harvesting rather than starved first. Table 4 gives the recovered activity (triplicate averages) as the absolute rate of substrate conversion on a protein basis, assuming that all of the optical density changes were due to the listed enzymes. Because repeated experiments with sterile flies were not done, it is not certain if any of the differences among them are significant. In order to compare these rates to those obtained with nonsterile flies, mean rates from nonsterile flies are

TABLE 4

*Enzyme activity rates for flies raised under sterile and nonsterile conditions**

	Aconitase	Guanase	Alcohol dehydrogenase	Alkaline phosphatase	Fumarase
Male†	134	47.3	71.0	15.8	1640
Female†	123	44.8	59.4	20.1	2170
Male+female‡	140	40.7	54.9	13.2	1707

* Rates are given as mμ moles of substrate converted/min/mg of protein.
† Sterile, each rate is the average of three readings.
‡ Average rates from nonsterile flies.

given in Table 4. The latter rates are averages of all the determinations in the six comparisons made between nonsterile diploid males and females.

Other enzyme activities: The Drosophila activities chosen for the present study were selected from among several previously unreported activities which were detected in supernatants derived from nonsterile flies. These activities were assayed by conventional techniques (COLOWICK and KAPLAN 1955) and included phenolphthalein glucuronidase, phosphodiesterase (using bis(*p*-nitrophenyl) phosphate as substrate), glycylglycine dipeptidase, rhodanese and β-glucosidase.

DISCUSSION

The methods used to obtain these results have several shortcomings. The Drosophila strains used were not isogenic and, indeed, the flies compared sometimes differed by visible mutations used as markers. However, no systematic enzyme activity differences were recorded except for male-female fumarase activity. This difference appeared in every comparison and in three unrelated crosses so was probably not due to uncontrolled strain or point mutation differences. It is very unlikely, too, that differences due to extra chromosomes existed in the other comparisons but, fortuitously, were exactly nullified by other mutational differences and were therefore overlooked. The evidence indicates, rather, that marker and other mutations had very little effect on the activities observed. FOX, MEAD and MUNYON (1959) observed similar independent behavior of the sex-peptide difference which they reported.

It is possible that microorganisms in the gut contents of the flies contributed to the measured rates. The gut contents, especially after starvation, were small compared to the masses of the flies and, being present in all flies, their effects tended to cancel out when the flies were compared. The activities recovered from sterile and nonsterile cultures are not directly comparable because differences in aging and conditioning of the flies could have influenced the results. However, inspection of Table 4 indicates that the activities recovered from the two kinds of culture were of the same order of magnitude. These considerations suggest that contributions from gut contents were probably negligible and, especially, could not account for the differential fumarase activities observed among the genotypes compared here.

It is not certain whether the fumarase activity difference was due to a qualitative or quantitative difference in enzyme content, or to enzyme inhibitors or enhancers. In fact, all of the activities measured may have been composites of several enzymes acting together. However, although still poorly identified, there were apparently differences and similarities of an enzymatic nature among the genotypes examined.

Only one set of determinations was made for triploids and two sets for superfemales so statistical comparisions are not meaningful for these genotypes. Inspection of the data (Table 3), however, reveals that the measured values for triploids and superfemales are very close to the control diploid values. Most of the differences are less than five percent for the relatively accurate assays of guanase, alcohol dehydrogenase and alkaline phosphatase activities and none of the recorded differences are large enough to indicate real differences between the three kinds of females.

It is unlikely that the enzyme levels from triploids and superfemales remained stable because their extra genes did not contribute to metabolism. Previous studies (cited above) have shown that polyploidy and hyperploidy nearly always affect the Drosophila phenotype. The stabilities support, rather, the presence of mechanisms in the cellular biochemistry which act to regulate enzymatic activities to physiologically determined levels. The consistent male-female activity ratios obtained in this study indicate that such levels do exist in Drosophila under these experimental conditions.

Some details of metabolic regulation of enzyme induction and regulation are already known in microorganisms. VOGEL (1957) elaborated a scheme whereby the rate of enzyme synthesis is regulated by the feedback of end products following from the enzyme's action. Further experiments may show these Drosophila activities to be similarly regulated, the stabilities reflecting control of the intracellular enzyme concentration. Of course, the end product control might be exercised on the activities of the enzymes as well as on their synthesis.

Any such stabilizing system, obviously, tends to obscure changes at the genic level and confuse interpretations of phenotypic effects. Numerous studies on Drosophila and other organisms have shown that mutations at one or sometimes several loci can cause enzyme activities to be altered or missing (HOROWITZ and FLING 1956). Some of these enzyme changes followed from enzyme molecules that were altered beyond compensation. Others resulted from mutations at loci not directly concerned with fixing enzyme specificity but concerned, rather, with other components of the enzyme synthesizing or regulating systems (PARDEE, JACOB and MONOD 1959; HOROWITZ, FLING, MACLEOD and SUEOKA 1960).

It is noteworthy that triploid females attained typical diploid activity levels despite a 50 percent increase in all of the genes concerned with these activities. This is reasonable as all of the genes related to control mechanisms were increased proportionately. Apparently, there were no threshold effects which could overcome such mechanisms.

Superfemales would be more likely to show changes because any regulatory

system originating on both the X and the autosomes would be unbalanced by the presence of three X chromosomes. The observed stability of the superfemale activities indicates that such systems do not exist for these five activities. Natural selection would tend to eliminate these systems in order to compensate for the usual sex chromosome differences, and their absence is compatible with the relative tolerance (BURDETTE 1940) with which Drosophila can accommodate changes in X chromosome material. Regulating systems for the fumarase activity may be localized on the X chromosome, but the data suggest that the observed male-female difference is more likely to follow from the sexual differentiation than from changes in X chromosome dosage. A projected comparison of normal males and diplo X, *tra/tra* males should illuminate the latter consideration.

It is difficult to predict what influence on the activity levels might be expected from Y chromosome changes, although the Y has been implicated in RNA metabolism (TRAVAGLINI, LEVENBOOK and SCHULTZ 1958). However, no large differences were recovered in the single experiment that was done to test the Y chromosome.

SUMMARY

Five enzymatic activities were measured in *Drosophila melanogaster* homogenates. Diploid males gave the same specific activities as diploid females in assays for aconitase, guanase, alcohol dehydrogenase and alkaline phosphatase, but gave fumarase activity levels that were significantly below those obtained from the females. Superfemales and triploid females gave specific activities for the five activities that were not significantly different from diploid female levels. No influence of the Y chromosome was detected on these five activities. The data indicate that some accurate physiologically determined enzyme levels are characteristic for Drosophila, and that homeostatic mechanisms act to preserve these levels despite changes in gene dosage.

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NEGATIVE CORRELATION BETWEEN RATE OF DEVELOPMENT AND FEMALE FERTILITY IN DROSOPHILA MELANOGASTER[1,2]

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THE fitness of an organism is the resultant of many components. Every character, even the most trivial, may contribute to the total fitness through one or more of the many biochemical pathways in the developmental process. These pathways are interrelated in an exceedingly complicated way. Yet the characters can be grouped into categories even if the interrelationships of the components within a category are not understood.

In this study rate of development (time from egg laying to eclosion) and fertility (number of progeny per adult female) were measured. The developmental rate is a major component of preadult fitness, and fertility the most important part of adult fitness.

BONNIER, JONSSON and RAMMEL (1959) have shown a high correlation between preadult viability, as measured by survival through the preadult stages, and the rate of development. Therefore a study of developmental rate also gives information about viability.

Preadult viability and female fertility have been studied in some detail in heterozygotes for second chromosome lethals in *D. melanogaster* (HIRAIZUMI and CROW 1960). Since the purpose of that work was simply to estimate the amount of inferiority of lethal heterozygotes for several fitness components, the data were not sufficient to make a detailed analysis of their interrelation. However it is interesting to note that in lethal heterozygotes these two components were positively correlated (although not statistically significant) while in the normal controls the correlation was significantly negative.

The purpose of the present study is to analyse this point as accurately as possible.

MATERIALS AND METHODS

The materials used in the present study came mainly from two sources.

1. Two sib-mated lines: (A) Madison Oregon-10 (abbreviated as M in the following). This line was isolated by the author from f Oregon stock (SEIGER 1958) but had no forked phenotype. Starting from a single male, chromosomes I, II, and III were made homozygous in the usual way by crossing to a multiple balancer stock (Basc; *SM1/Pm*; *Ubx*^{130}*/Sb*) to form the M Oregon-1

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line. Because of the simultaneous presence of inversions on three chromosomes there may have been some crossovers. However, after its construction this line was kept by sib-mating for ten generations before starting the present study. (B) 3V-30. This line was made homozygous for chromosomes I, II, and III by DR. SEIGER, starting from a single male which was captured at Vandeventer Flats. Since that time this line had been kept by sib-mating for 30 generations.

2. Selected lines for fast and slow rate of development: These were lines free of second chromosome lethals from the previous study (HIRAIZUMI and CROW 1960) which had been kept in our laboratory for about three years in small mass cultures. Twenty lines were chosen at random and mixed together, then kept for seven generations in one large mass culture without any conscious selection. Nineteen pairs were then chosen for the selection experiment.

Among the 19 culture vials two, one showing the fastest and one showing the slowest average developmental rate, were chosen to start the fast and slow lines. About ten pairs which eclosed on the very first day were then chosen from the F_1's of the fastest culture. This process was repeated twice, inserting two generations of mass transfer without selection between the selected generations. Therefore there was a total of three generations of selection for the fast line. Since, as will be seen later, heterozygotes generally show faster development than homozygotes, there may have been some selection for heterozygosity. A similar selection procedure was followed for the slow line, except that it continued for five generations. Since the fertilities of the pairs eclosed at the end of a culture were generally very low (often they were completely sterile), pairs to continue the selection were chosen from flies eclosed one or two days before the last ones emerged. Therefore the selection intensity might be slightly weaker for the slow line than for the fast.

Starting from these selected lines and using the multiple balancer stock, one fast and two slow developmental rate lines homozygous for the second and third chromosomes were established (19A for the fast line, 12A and 12B for the slow lines). Mating schemes were designed to replace the sex chromosomes by those of the M line. The fourth chromosome was entirely ignored in this study on the assumption that its contribution, if any, would be very small.

Since a slight change in environment may cause a substantial change in the rate of development, a trial to get relatively comparable culture conditions was made in the following way: One (sometimes more) fertilized female(s) of the genotype in question and one fertilized *cn bw* female were put together into a food vial and allowed to lay eggs for 24 hours. Males were, of course, discarded before the tests to avoid undesired matings.

Counts were made twice a day until all the flies had eclosed. The average time from egg laying to eclosion was then computed for both genotypes separately in the two sexes, and only those cultures in which the unweighted average eclosion time of the *cn bw* males and females lay between 9.00 and 10.50 days were selected for the analysis.

In order to minimize the effects of environmental differences, the following

analysis was used. Let x and y be the eclosion time of the *cn bw* strain and of the genotype in question, taking the unweighted average for the two sexes. Since the value of x was variable within the chosen interval 9.0 to 10.5, it is necessary to adjust the y value to some constant x within this range. For this purpose the linear regression of y on x was computed for each genotype, and the average developmental rate of y was estimated at the point $x = 9.80$. The averages of x for almost all genotype sets were close to this value, and this was chosen because the standard error of the estimate of y is the smallest at the average of x.

Female fertility was measured as follows: One female of age less than 12 hours was put into a food vial together with three Canton-S males between one and two days old. Every three days they were transferred to a fresh food vial for three changes. Therefore each female was allowed to lay eggs for nine days, and then the adult progeny were counted. The tests were replicated at several different times. At each time the M line was included in each group to check the effect of possible secular environmental fluctuations but since there was no, or very little, environmental effect, all the data were treated as if they were obtained at the same time.

Maternal effect: Several crosses were made reciprocally to detect a possible maternal effect on the rate of development and female fertility. The results are summarized in Table 1. In all but one (cross No. 5 in fertility) there was no significant difference between the reciprocal crosses. In cross No. 5 the difference was significant but a true maternal effect is quite unlikely in this case because the F_1 females were less fertile when they were the descendants of the more fertile mother. This difference might be due either (1) to a somehow poor culture condition from which the tested F_1 females were collected or (2) merely to chance which might produce apparently significant results among so many tests.

Thus there is so far no demonstrable maternal effect when each set of reciprocal crosses is examined separately, but still there may be a significant difference when all the data are pooled. The sum of the eclosion times of the F_1 genotypes whose mothers showed a faster rate is 46.01 ± 0.14 compared with 46.10 ± 0.15 for the reciprocal crosses which is not significant difference. In fertility, the total of F_1 females whose mother showed higher fertility is 3625 ± 50 while that of the reciprocal cross is 3691 ± 55, which also is not significantly different. Therefore we conclude that there is extremely little, if any, maternal effect.

Interaction between chromosome II and III: For further analysis it is necessary to know how the individual chromosome pairs contribute to a fitness component. To answer this question we first seek an approximate but general rule which the majority of chromosome pairs obey, then proceed, if necessary, with various modifications, to improve the accuracy of the approximation. This can be done by examining the amount and direction of deviations of the observed results from the values which are expected based on the first approximation rule.

There are two possible simple rules, (1) complete additivity and (2) completely multiplicative contribution from each chromosome pair. When the dif-

TABLE 1

Time of eclosion and female fertility in reciprocal crosses. M, 3V, and 12B are chromosomes described in the text. pI is P_1-I *Oregon* (SEIGER *1958*)

Cross no.	Parents Chromosome constitution						Offsprings		
	Female			Male			Female		Male
	I	II	III	I	II	III	Time of eclosion	Fertility	Time of eclosion
1	M	M	M	3V	3V	3V	9.09±0.09	545±12	.....
	3V	3V	3V	M	M	M	9.09±0.04	568±13	.....
2	M	M	M	pI	pI	pI	9.67±0.09	364±25	.....
	pI	pI	pI	M	M	M	9.59±0.05	420±19	.....
3	pI	pI	pI	3V	3V	3V	8.96±0.06	531±19	.....
	3V	3V	3V	pI	pI	pI	9.02±0.09	526±20	.....
4	M	M	M	M	M	3V	9.10±0.03	509± 9	9.28±0.04
	M	M	3V	M	M	M	9.06±0.06	484±10	9.25±0.05
5	M	M	M	M	3V	3V	.....	498±14**	.....
	M	3V	3V	M	M	M	.....	581± 5	.....
6	M	M	M	M	12B	M	.....	374±12	.....
	M	12B	M	M	M	M	.....	314±38	.....
7	M	M	M	M	M	12B	.....	468±17	.....
	M	M	12B	M	M	M	.....	455± 5	.....
8	M	M	M	M	12B	12B	.....	336±25	.....
	M	12B	12B	M	M	M	.....	343±23	.....

** This difference is significant at the one percent level.

ferences among the chromosome in question are very small these two rules give approximately the same expectations, but if the differences are large the first sometimes gives biologically meaningless expectations, such as negative fertility, while the second always remains meaningful. As a matter of fact, as will be seen later, the chromosome pairs obey the multiplication rule somewhat closer than the addition rule, and hence this is the candidate for the first approximation rule. For a statistical test of goodness in fit, it is more efficient to transform the observed values into logarithms, which converts the multiplication into addition. Normality tests were applied to the distributions with the two scales, but neither deviated significantly from the normal distribution. However, since the logarithmic transformation appeared to make the distribution somewhat less normal in the female fertility distribution, and since the observed values are easier to understand biologically, only the original untransformed values are presented

TABLE 2

Time of eclosion and female fertility in various combinations of chromosome II and III

Designation	Homozygote				Heterozygote			
	Chromosome II	Chromosome III	Time of eclosion	Female fertility	Chromosome II	Chromosome III	Time of eclosion	Female fertility
A	M/M	M/M	9.64±0.06	397±12	M/M	M/M	9.64±0.06	397±12
B	19A/19A	M/M	9.42±0.04	308±18	19A/M	M/M	9.28±0.09	462±11
C	M/M	19A/19A	9.50±0.07	280±16	M/M	19A/M	9.44±0.05	460± 4
D	19A/19A	19A/19A	9.31±0.05	220±24	19A/M	19A/M	9.01±0.05	497±15
	A × D		89.75±0.70	87.3±9.8			85.86±0.67	197.3±8.9
				×10³				×10³
	B × C		89.49±0.75	86.2±7.2			87.60±0.99	212.5±5.4
B	3V/3V	M/M	9.39±0.12	312±21	3V/M	M/M	9.03±0.06	430±24
C	M/M	3V/3V	9.48±0.04	261±16	M/M	3V/M	9.18±0.04	496± 7
D	3V/3V	3V/3V	9.05±0.08	169±24	3V/M	3V/M	9.05±0.06	542± 7
	A × D		87.24±0.95	67.1±9.9			**87.24±0.76	215.2±6.7
				×10³				×10³
	B × C		89.02±1.17	81.4±7.3			82.90±0.64	213.3±12.4
B	12A/12A	M/M	9.83±0.09	162±17	12A/M	M/M	9.05±0.14	361±29
C	M/M	12A/12A	10.13±0.12	74±13	M/M	12A/M	9.37±0.04	462±13
D	12A/12A	12A/12A	10.10±0.35	41± 7	12A/M	12A/M	9.12±0.16	457±17
	A × D		97.36±3.42	16.3±2.8			87.92±1.63	181.4±8.7
				×10³				×10³
	B × C		99.58±1.51	12.0±2.5			84.80±1.33	166.8±13.3
B	12B/12B	M/M	9.32±0.04	168±32	12B/M	M/M	8.95±0.13	346±19
C	M/M	12B/12B	10.05±0.21	246±29	M/M	12B/M	9.09±0.05	461± 8
D	12B/12B	12B/12B	9.60±0.08	144±17	12B/M	12B/M	8.61±0.13	340±17
	A × D		92.54±0.94	57.2±6.9			83.00±0.63	*135.0±8.0
				×10³				×10³
	B × C		93.67±2.02	41.3±9.4			81.36±1.23	159.5±9.1

* Significant at the five percent level.
** Significant at the one percent level.

in Table 2. In fact, the conclusions are the same whether based on transformed or raw data.

Let A and D be the measures for the two original combinations of chromosome II and III, B and C be the measures for the two genotypes obtained by exchanging the two chromosome pairs reciprocally. Then under the rule of multiplicative action, $BC - AD = 0$. In two cases this value was significantly different from zero but since there were 16 cases examined (eight cases for developmental rate, eight for fertility) this deviation might well be expected merely by chance alone.

There was, however, an apparent over-all tendency. With low values of BC, AD tends to be greater than BC. This was tested by computing the correlation between BC and $(BC - AD)/BC$. The correlation was positive and significant for developmental rate ($P < 0.05$) but not for female fertility.

Although there is therefore some evidence for interaction of chromosomes, this may be due to metrical bias. In any event the amount of interaction is small and may be neglected in the further analysis without serious error.

Relation between developmental rate and female fertility: We shall proceed as if there is no interchromosome interaction. Then we can estimate the contribution of each tested chromosome from two independent observations. For simplicity, the values of the chromosomes II and III from all crosses were measured relative to the M line. The relative level of the tested chromosomes II and III was measured by taking the square root of BD/AC and CD/AB respectively. The estimates for eclosion time thus obtained were always smaller in chromosome II's (i.e., development was faster) than that in the chromosome III's. This suggests that the chromosome II of strain M contributes more to slow development than its chromosome III does. Since the average of this difference was about 0.010, we assign the value 1.005 and 0.995 to the chromosomes II and III of strain M respectively. The contribution to the relative developmental time of each tested chromosome was re-computed on the basis of these values. No correction was made for fertility because there was no such clear difference between chromosome II and III. We therefore assign the value 1.00 for both chromosomes II and III of M line. After making these corrections we obtain the results given in Table 3.

When the developmental rate of the chromosome is faster (i.e., eclosion time less) than the level of 1.000 the correlation between the time of eclosion and female fertility is significantly positive ($r = +0.92$, $n = 4$, $P \leq 0.01$). Since the larger value in the time of eclosion means the slower rate of development, positive correlation means that the developmental rate is negatively correlated with female fertility. But when development is slow the correlation becomes negative though not significant ($r = -0.45$, $n = 2$, $P > 0.05$ for the four items at the bottom of Table 3).

The former relation is also true in the heterozygous condition, ($r = +0.800$, $n = 6$, $0.02 < P < 0.01$).

Thus the negative correlation between female fertility and developmental rate when the latter is fast is now established.

TABLE 3

Relation between time of eclosion and female fertility for the contribution of various chromosomes relative to the M chromosome

Homozygous condition			Heterozygous condition with M		
Chromosome	Time of eclosion	Female fertility	Chromosome	Time of eclosion	Female fertility
M (II)	0.995	1.00	.....	....	...
19A (II)	0.984	0.78	19A (II)/M (II)	0.963	1.12
19A (III)	0.982	0.71	19A (III)/M (III)	0.970	1.12
3V (II)	0.969	0.71	3V (II)/M (II)	0.966	1.09
3V (III)	0.969	0.60	3V (III)/M (III)	0.972	1.25
12B (II)	0.966	0.50	12B (II)/M (II)	0.943	0.80
M (II)	1.005	1.00	.....	....	...
12A (II)	1.013	0.48	12A (II)/M (II)	0.961	0.95
12B (III)	1.031	0.73	12B (III)/M (III)	0.947	1.07
12A (III)	1.034	0.22	12A (III)/M (III)	0.984	1.22

DISCUSSION

The data obtained earlier for another study (HIRAIZUMI and CROW 1960) can also be used to measure the relation between developmental rate and fertility. The correlation coefficient for normal second chromosomes in the heterozygous condition is $+0.600$ ($P = 0.02$), in agreement with the present results. Thus in two independent studies in highly viable populations a negative correlation between two principal components of fitness is shown to exist. This has been expected on theoretical grounds (ROBERTSON 1955) and has been discussed by several writers (see for example, FALCONER 1960).

On the other hand the correlation becomes positive when less fit genotypes are studied. One line of evidence comes from the fact that lethal heterozygotes as a group are low in both fertility and rate of development (HIRAIZUMI and CROW 1960). This same study showed a positive though not significant correlation ($r = -0.225$) between fertility and developmental rate of individual genotypes, each heterozygous for a different lethal.

The data in the present study are in agreement, though the numbers are too small for statistical significance. They do show, when taken at face value, a positive correlation between the two components when both are at a low level.

Natural selection favouring intermediate phenotypes has been discussed theoretically in various places but no consistent experimental evidence has been given on how it operates. The present study may give a hint for this question. The two major components are negatively correlated within a certain range of values. In natural populations the maximum fitness combinations of these two components must be located somewhere in this high range. A reduction in one character involves an increase in another. Thus they somehow compensate in a certain range but beyond it an increase in one component may become insufficient to compensate for a reduction in the other resulting in a decline in the total fitness.

Hence the more fit genotypes must have an intermediate level in both components.

Assuming a linear relationship between the two components and independent contribution of chromosome pairs (these assumptions may be approximately true within a relatively small range of their levels) the combinations of two chromosomes which show similar levels in developmental rate give higher fertility, hence the combinations of similar levels must generally have a selective advantage and will be accumulated in a population.

In natural populations of Drosophila the generation times overlap, and this is especially important during the warm season when the population size increases rapidly. For such a species fitness is best estimated in terms of FISHER's Malthusian parameter, m, (FISHER 1930) which is defined as a real root of the following equation.

$$\int_0^\infty l_x b_x e^{-mx} dx = 1$$

Here l_x is the proportion of survivors to the age x, b_x is the average number of offspring per individual during the age interval dx. In Drosophila l_x is measured by the proportion of fertilized eggs which survive to the age x. Since the proportion of survivors after eclosion remains nearly 100 percent for the most important first two or three weeks (HIRAIZUMI and CROW 1960) l_x may be taken as unity through this period (this excludes the larval stage). The number of female progeny per parental female at age x gives a measure of b_x. The lower limit of integration is chosen as the age when a genotype in question reaches adulthood. A good measure for this is the time from egg laying till eclosion.

It is of interest to compare the contribution of fertility and rate of development in determining total fitness. In Drosophila cultures the death rate is low during early adult life, and the egg laying is roughly constant during the first two or three weeks. Therefore we take $b_x = c$ for the interval $14 \leq x \leq 33$ days. Thirty-three days is taken arbitrarily because fertility was measured over this range in the earlier experiments. However, the upper limit of the integration is of little consequence in determining the measure of fitness. We also take $l_x = (1-s)$ to include the larval stage mortality, where s is a measure for the death rate at this stage.

Then the Malthusian parameter, m, is given by

$$c(1-s) \int_{14}^{33} e^{-mx} dx = 1$$

or roughly

$$c(1-s)e^{-14m} = m$$

A simple example will give us an understanding of how much the two factors contribute to the total fitness.

Let us consider four model cases.

		$c(1-s)$	K
Case 1	a	5.00	11.4
	b	5.25	12.0
Case 2	a	5.00	13.3
	b	5.25	14.0
Case 3	a	5.00	15.2
	b	5.25	16.0
Case 4	a	2.50	15.2
	b	2.625	16.0

where $c(1-s)$ is the survival and fertility factor and K is the mean days from egg to adulthood. In all four cases K and $c(1-s)$ are smaller (i.e., faster development but less fertile) in a than in b by five percent. The m value for each case was computed approximately, and the values are listed in the following table.

		m	c'	$(c'-c)/c \times 100$
Case 1	a	0.2595	.....	.....
	b	0.2536	5.84	16.8%
Case 2	a	0.2311	.....	.....
	b	0.2247	5.87	17.4%
Case 3	a	0.2092	.....	.....
	b	0.2027	5.95	19.0%
Case 4	a	0.1748	.....	.....
	b	0.1703	2.87	14.8%

where c' is the value of $c(1-s)$ in b to give the same m value as in a when K remains the same, and $(c'-c)/c \times 100$ is the percent increase in $c(1-s)$ to com-

pensate for a five percent reduction in the developmental rate. It is clear from the table that it is necessary to increase the fertility factor by 15 to 19 percent to overcome a five percent reduction in the developmental rate. This required percent increase becomes larger as K gets larger with the same fertility (cases 1 to 3). In other words when the life cycle of the flies goes slowly, the developmental rate becomes more important relative to the fertility. The comparison between cases 3 and 4 shows that when the fertility factor is small, fertility is more important relative to the developmental rate. All these relations may be much more complicated in natural populations, but in an organism like Drosophila with early maturity and long reproductive time the developmental rate is an important part of the total fitness.

SUMMARY

The relation between the rate of development and female fertility was studied for chromosomes II and III of *D. melanogaster*. The principal conclusions were as follows:

1. There was no detectable maternal effect in the two components.
2. To a first approximation chromosome II and III contributed to each component in a simple multiplicative fashion although significant, but small, deviations from this rule were observed.
3. The rate of development was negatively correlated with female fertility when the developmental rate was faster than a certain level but positively when it was slower than this level.
4. Some possible meanings of this negative correlation in natural populations, and the relative importance of developmental rate in determining the total fitness were discussed.

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MUTATION STUDIES AT THE A_1 LOCUS IN MAIZE. I. A MUTABLE ALLELE CONTROLLED BY Dt[1]

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THE gene A_1 in maize was first reported, with its mutant allele a_1, by EMERSON (1918, 1921). In these reports and later publications by EMERSON and ANDERSON (1932) and LAUGHNAN (1948) the A_1 allele found in North American races was shown to be necessary for the production of anthocyanin pigments in various tissues of the corn plant. With the complementary genes A_2, *B*, *Pl*, and R^r or r^r, A_1 produces a deep purple pigmentation of the sheath, brace roots, auricle, leaf margin, husks, tassel, and anthers; with A_2, *Pr*, *C*, and *R*, it produces a purple pigment in the aleurone. With *P* it produces a bright red pigment in the pericarp. When a_1 is homozygous in the above genotypes, it causes the substitution of a brown pigment for purple in all tissues where anthocyanin normally appears, except for the aleurone, where there is no pigment. Various allelic combinations of the above genes can alter the type of pigment produced and the regions in which pigment is expressed, giving sun red, brown and green plants depending upon the genotype involved.

The *A* locus is in linkage group 3 between lg_2 and *et* with 33 crossover units separating lg_2 and A, and 12 units separating *A* and *et*. It has been placed cytologically in the distal one fifth of the long arm of chromosome 3 (MCCLINTOCK 1931).

The mutant *a* which behaves as a normal stable recessive was shown by RHOADES (1938, 1941) to be mutable in the presence of the dominant factor *Dt*. This factor, located near the end of the short arm of chromosome 9, causes *a* to mutate to *A* in all the tissues where *A* vs. *a* differences can be seen. In the proper genetic background, the mutant areas appear as small dots of *A* tissue on a colorless aleurone, and as long narrow purple sectors on the brown sheath, culm, husks, etc. These sectors may extend into the tassel to give purple anthers which, depending on whether or not the sector includes the germ line, may transmit a mutant *A* allele in 50 percent of the pollen.

There are known, at the present time, many A_1 alleles in addition to those reported by EMERSON in his original study. Most of these have arisen as mutants from the four original parent alleles *A*, *a*, A^b and a^p and have been organized into several groups according to their phenotype, parentage, and mode of origin (RHOADES 1941; LAUGHNAN 1949; NUFFER 1952).

The compound nature of certain alleles of South American origin has been demonstrated by LAUGHNAN (1949). These alleles characterized as A^b have at

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least two components, α (dilute anthocyanin in seed and plant and dominant brown pericarp) and β (strong anthocyanin in seed and plant and recessive red pericarp) which can be separated by crossing over to produce single unit alleles. The original a^p allele apparently is an α isolated by crossing over or by mutation of β. Further subdivision of these units is suggested by recent investigations (LAUGHNAN 1956).

The common allele in North American races *(A^r)* appears to be a single unit with a phenotype that closely resembles the isolated β component of A^b. The remaining one of the four original parent alleles *(a)* apparently arose as a mutant from *A*. Although it is phenotypically at the null level it retains its pairing affinity for all the known components of the A_1 locus and it has the property of reverting back to *A* in the presence of *Dt*.

With the increasing complexity of the *A* locus, both as to its structure and the diversity of its mutant alleles, there arises a serious problem of terminology. Not only are there many mutant types, but they are involved in successive mutational steps whose sequence is important. For this reason, any notation of these alleles should include, besides the descriptive symbol, a designation of the parent allele and the mode of origin. The following is a variation of the system used by L. J. STADLER in his research and various publications:

The notation of each allele requires the base symbol *(A)*, which has been previously used in the literature. An additional descriptive symbol is added as a superscript. Thus A^{br} describes the *A* allele with recessive brown pericarp. Following this descriptive symbol and separated from it by a colon is a distinctive name, if the allele is not a mutant, or its origin if it is a mutant. The only alleles of unknown origin are A^b, *A*, a^p, and possibly *a*. Accessions of these alleles are each given a distinctive name after the allele has been purified by extracting from a heterozygote with a distinguishable allele. For example, various *A* alleles used here are designated *A:Cache*, *A:Quapaw*, etc.

The mutants from a particular allele are numbered in consecutive order in a single series such as *a-1*, *a-2*, *a-3:A Cache*. As further study reveals more about these mutant alleles, they may be distinguished as a^m-*1*, *a-2*, a^s-*3*, etc., without changing their number (the symbol a^s signifies the null level, an allele which will not mutate back to the dominant form in the presence of *Dt*).

If there has been some agent such as *Dt* causing the mutation, then this fact is added as a descriptive capital letter immediately preceding the numerical designation of the mutant. Therefore, the mutant A^{br} from *a* by the action of *Dt* is A^{br}-*D4:a*. The designations of the various origins are: *D* for *Dt*, *U* for ultraviolet, *X* for X ray. When the mutant arose spontaneously or from unknown causes this symbol is omitted.

In actual use these designations can be shortened to include only the allelic symbol and its serial number, as *A–D4*, a^m-*1*, a^s-*3*, etc.

Origin of a^m-1: On a selfed ear of a plant carrying *A:Cache/a*, *Dt Dt* there occurred a single seed which was colorless and heavily dotted (the ear segregated 3 colored:1 colorless dotted seeds). It had more than 1,000 dots as compared to

FIGURE 1.—Ear from a^m *a*, *Dt Dt* selfed showing a^m in one, two, and three doses compared to *a* in three doses, all with three doses of *Dt*.

the colorless sibs (*a a a* in 3n endosperm) which averaged only 58 dots per seed. The plant grown from this seed produced a selfed ear (Figure 1) that had 26 normal *a* dotted and 93 very heavily dotted seeds. Subsequent tests proved the case to be a highly dottable allele of *a*, which like *a* was unstable in the presence of *Dt* but completely stable in its absence.

Further mutation trials with both the parental alleles, *A:Cache* and *a*, have shown that the former yields additional cases while the latter does not. It is concluded that a^m*-1* came from *A:Cache* and it is therefore, tentatively designated a^m*-1:Cache*.

Comparison with a: In the absence of *Dt* there is no apparent difference between *a* and a^m, both producing a colorless aleurone and brown plant. In the presence of *Dt* however, striking differences are seen. These can best be described by comparison of a^m with *a* in the sib progeny from the cross *a a*, *dt dt* × a^m *a*, *Dt Dt*. The two classes of seeds seen on the resulting ear have endosperms (3n) of the constitutions *a a a*, *Dt dt dt* and a^m *a a*, *Dt dt dt*. The number, size and appearance of the a^m vs. *a* dots are clearly demonstrated in the aleurone layer of these endosperms (Figure 2). Certain aspects of *a* dotting however, are more clearly seen on seeds from the cross *a a*, *Dt Dt* × a^m *a*, *Dt Dt* (Figure 3).

The most obvious difference between the two alleles is in the frequency of mutation (number of dots). Counts of the dot number in all the seeds from nine ears of the cross of a^m *a Dt Dt* on different *a a dt dt* cultures are listed in Table 1. This material has one dose of *Dt* in each cell. The first class (*a a a*) has an average of 0.73 dots with a range of zero to six dots on each seed. The second class on the other hand, has an average of 98 dots with a range of nine to 250 dots on each seed. (The difference between a 6-dot *a a a* seed and a 9-dot a^m *a a* seed is clear because of distinctive differences in the type of dots.) These averages are not entirely comparable as they represent a comparision of *a a a* with a^m *a a*. Because of this and because of other differences these averages can be made more

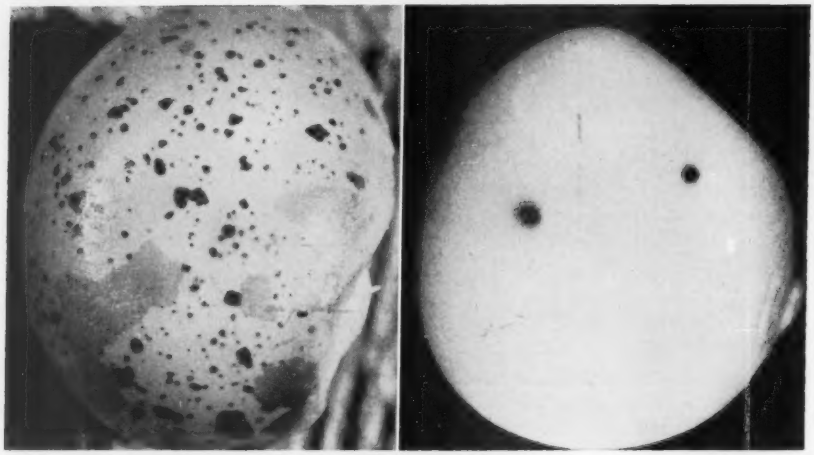

FIGURE 2.—Comparison of $a^m\ a\ a$, *Dt dt dt* (left) vs. *a a a*, *Dt dt dt* (right) on sib seeds from a cross of *a a*, *dt dt* × a^m *a*, *Dt Dt*.

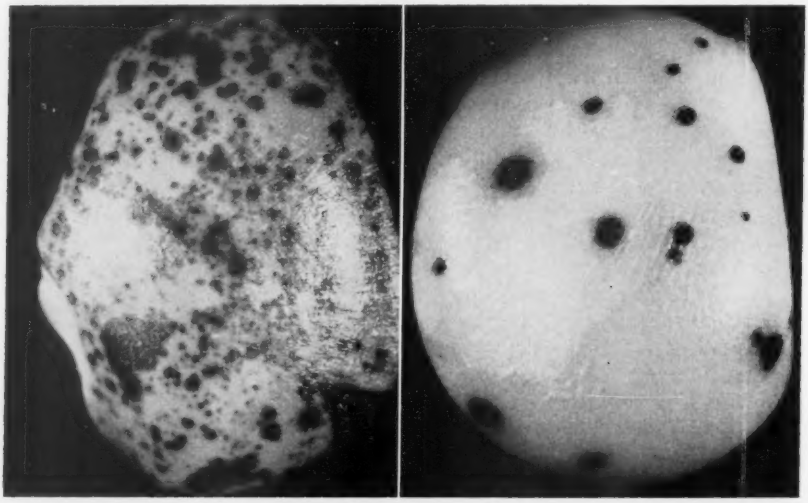

FIGURE 3.—Comparison of $a^m\ a\ a$, *Dt Dt Dt* (left) vs. *a a a*, *Dt Dt Dt* (right) on sib seeds from a cross of *a a*, *Dt Dt* × a^m *a*, *Dt Dt*.

meaningful with the following corrections: RHOADES (1941) has shown that two and three doses of *a*, in the presence of *Dt*, produce approximately two and three times as many dots as one dose. We have also found this to be true, therefore to make these two classes comparable we must correct the counts by subtracting

TABLE 1

Comparison of a *vs.* a^m *dot frequency from a cross of* a a, dt dt *by* a^m a, Dt Dt

Plant	No. *a* seed	Total dots	Av. dots	No. a^m seed	Total dots	Av. dots	a^m/a
66:501.1-1	36	22	0.6	53	5,966	112.5	187.5
66:526-6	51	5	0.1	38	673	17.7	177.0
67:626.1-8	15	16	1.1	19	1,776	93.4	84.9
.1-10	29	18	0.6	22	1,575	68.9	114.9
.2-3	15	23	1.5	23	3,430	149.1	99.4
67:660-4	48	69	1.4	50	8,065	161.3	115.2
67:626.1-3	15	9	0.6	19	1,175	61.8	103.0
.1-4	33	20	0.6	32	2,840	88.7	147.8
67:629.1-1	53	42	0.8	29	2,710	93.4	116.7
-5	45	23	0.5	35	3,217	91.9	183.8
Total	340	247	0.73	320	31,366	98.0	134.2

2/3 of the *a* class counts from both classes. Thus $0.73 - 2/3\ (0.73) = 0.24$ dots for each *a* gene per seed and $98.0 - 2/3\ (0.73) = 97.5$ dots for each a^m gene per seed. The frequency of a^m dots is, therefore, 97.5/0.24 or 406 times as great as *a*. This is misleading as a large portion of the a^m dots are very small indicating that they must have occurred at a later stage when the number of cells in the aleurone, and therefore the number of chances of mutation, for a given seed were much greater. A count of the dots of the *a* size (78-cell average) on a^m seeds reveals that at the stage when *a* mutates 0.24 times per seed, a^m mutates 16 times, or in other words, at a given stage, a^m mutates 67 times as frequently as *a*.

It will be noted that the frequency of 0.73 for the *a a a, Dt dt dt* seeds is considerably below the average of 2.7 dots, for this same genotype, found by RHOADES (1938). This is probably due to genetic modifiers, and perhaps due to the fact that RHOADES used a different allele which he describes in his report. Further discussion of the effect of modifiers of dotting will be found in a later report.

A second major difference is seen in the size of dot. Those on the *a* seeds at both *Dt* dosage levels (Figures 2 and 3) were comparatively uniform in size averaging 78 cells each and ranging from 25 to 560. (On other sib *a* seeds there were occasional extreme variants on each side but most grouped very near the mean.) The dots on the a^m seeds, however, ranged widely from single-cell to whole-seed cases with no tendency toward grouping at an average size. There was, however, a proportionate decrease in frequency per kernel as the size of the sector (dot) increased. This can be explained when one considers that each dot is a sector of mutant tissue composed of cells that have descended from a single cell in which a mutational change occurred, and that the earlier the mutational event occurs the larger will be the sector and the smaller will have been the population of cells among which such mutational events could have taken place. Thus it can be seen that *a* mutates most frequently at a particular stage while a^m apparently mutates at an even higher rate at all stages.

Two additional differences which must be considered together because they appear to be related are those of dot shape and intensity. The dots on the *a* seeds

are mostly round, uniform, intensely colored, and have a diffuse outline. Occasionally there may be found a single dot which is lighter in color and has a sharp outline. The dots on the a^m seeds include a few of the type just described (since the seeds are actually a^m *a a*) but mostly dots with a very sharp erratic outline and moderate to light intensity. The moderately intense dots usually have a single course of lightly pigmented cells representing a diffusion layer all the way around the sector. The irregular outline of the a^m dots appears to follow the developmental pattern of the aleurone cells more closely than does that of the *a* dots.

It appears, therefore, that the *a* dots are more symmetrical and diffuse in outline because they have more pigment which can therefore diffuse farther into the area of genetically colorless tissue giving a blending effect along their borders.

These last two differences can be equated to a more basic difference that is in the types of mutant alleles produced. RHOADES (1941) found that among 29 extracted *A* mutants from *a* through the action of *Dt*, 27 were A^r's (strong), one was A^{rb} (less strong) and one A^{br} (medium). In contrast it has been found that 23 unselected *A* mutants from a^m included two A^r, two A^{rb}, 13 A^{br} and six A^{lt} (weak).

These differences, described as they are expressed in the aleurone, are also projected into the other tissues of the plant and can be recognized when one understands the developmental pattern of the cells of the tissue being observed. Thus, whereas a few intense sectors occur in all potentially anthocyanin-producing tissues in *a a* plants, several times as many occur in the same tissues of a^m a^s plants (Figure 4).

Gene dosage: By appropriate crosses as pointed out by RHOADES (1941) it is

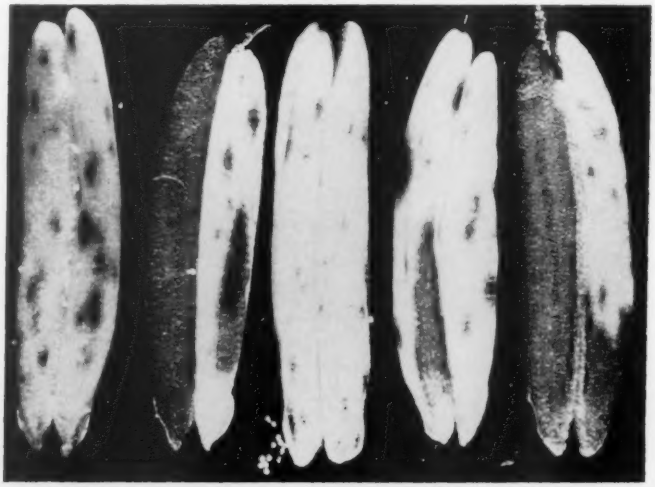

FIGURE 4.—Anthers from an a^m a^s, *Dt Dt*, R^r, *Pl* plant showing *A* sectors.

possible to manipulate the dosage of a particular gene in the 3n maize endosperm. For example, a cross of $a^s\ a^m$, *dt dt* × $a^m\ a^m$, *Dt Dt* can be made to produce ears which have seeds with one dose of a^m ($a^m\ a^s\ a^s$) and three doses of a^m ($a^m\ a^m\ a^m$). Similarly from $a^m\ a^m$, *dt dt* by $a^s\ a^m$, *Dt Dt*, ears with two doses ($a^m\ a^m\ a^s$) and three doses ($a^m\ a^m\ a^m$) of a^m can be produced. Dot counts on such seeds were made and it was found that the frequency for one dose was 114 dots per seed, for two doses 225 dots, and for three doses approximately 330 (an approximation, because seeds with more than 300 dots cannot be counted accurately. These counts show that there is a simple linear relationship between gene dosage and mutation frequency of a^m.

Similar crosses were made to study varying doses of *Dt* against an a^m background. They were $a^m\ a^m$, *Dt dt* × $a^m\ a^m$, *Dt Dt* which gave a comparison of one *vs.* three doses (Figure 5) and the reciprocal cross which gave a comparison of two *vs.* three doses of *Dt*. The counts were as follows: one dose, approximately 300 dots; two doses, 800 or more; and three doses well over 2,000. Accurate counts were impossible because of the overlapping and blending of dots. The two- and three-dose counts were probably on the conservative side. These counts suggest the exponential relationship reported for *Dt* dosage by RHOADES (1938, 1941).

Types of Mutants: As previously stated, plants with an $a^m\ a^m$ *Dt Dt* genotype produce frequent mutant sectors of various sizes and types, including those arising in germinal tissue. Whenever a mutational event occurs in a cell in the germ line 50 percent of the gametes produced from that cell's descendents will be mutant. This may occur either as a tassel or an ear sector, or if the event is late enough it may be expressed only as a single megaspore or microspore, in which case a single mutant seed will be produced.

By sampling ear and tassel sectors and collecting individual seeds (each representing a different occurrence) it has been possible to collect a sizeable

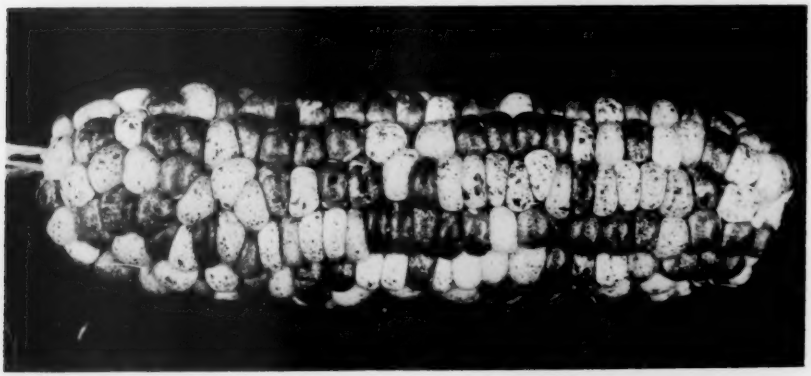

FIGURE 5.—Ear from the cross $a^m\ a^m$, *Dt dt* × $a^m\ a^m$, *Dt Dt* showing one *vs.* three doses of *Dt* on seeds with three doses of a^m.

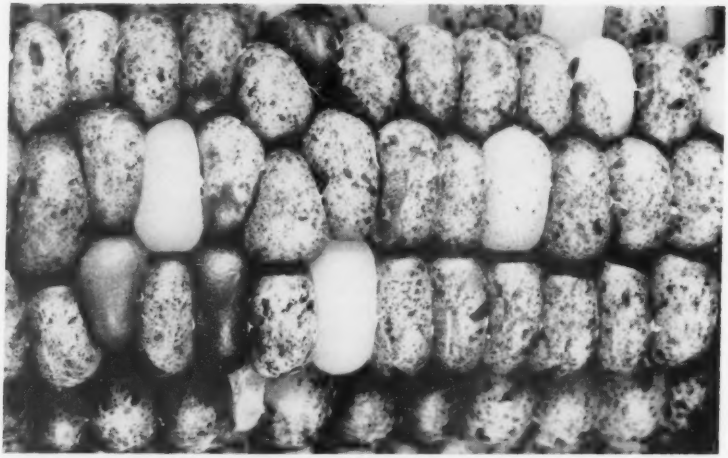

FIGURE 6.—Four mutant types from the cross $a^s\ a^s$, *Dt Dt* × $a^m\ a^m$, *Dt Dt*. Row 2 no. 4 a^s, 2 no. 10 a^{m-}, 3 no. 3 A^{lt}, 3 no. 5 *A*, and 3 no. 7 another a^s. The grouping of these mutants is by chance as a^m came from the male parent.

number of mutants including a wide array of types, several of which are demonstrated on an ear from the cross $a^s\ a^s$, *Dt Dt* × $a^m\ a^m$, *Dt Dt* (Figure 6). The grouping of mutants in this example is by chance since the a^m in this case comes from the male. This ear was selected for photographing because it carried an ideal collection of mutants. Other seeds from this and sib ears had partial seed sectors (Figure 7) that demonstrate several of the types of mutant alleles

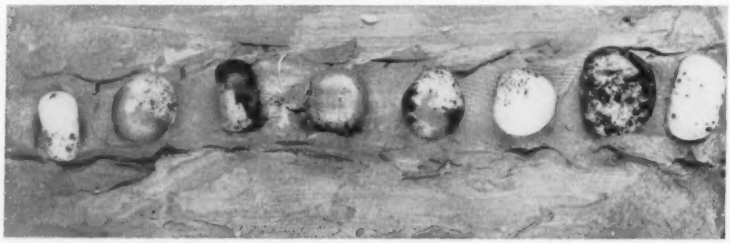

FIGURE 7.—Mosaic seeds from a cross of $a^s\ a^s$, *Dt dt* × $a^m\ a^m$, *Dt Dt*; no. 1 a^s, no. 2 A^{lt}-a^s, no. 3 *A*, no. 4 A^{lt}, no. 5 *A*-A^{lt}, no. 6 a^s. Numbers 7 $a^m\ a^m\ a^m$, *Dt Dt Dt*, and 8 *a a a*, *Dt Dt* are included for comparison.

produced. These when considered with the partial ear sectors (Figures 8 and 9) indicate the wide range in size (an expression of time of mutation) and type of mutational event.

The mutants from a^m fall into three major groups: (1) stable phenotypic

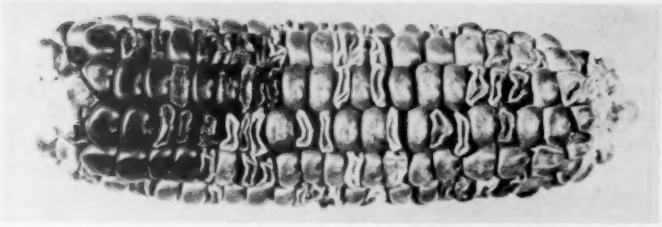

FIGURE 8.—A brown pericarp ear from the cross *a^m-Sh, Dt Dt, P* × *a-sh, Dt Dt* showing sector of A^r with red pericarp.

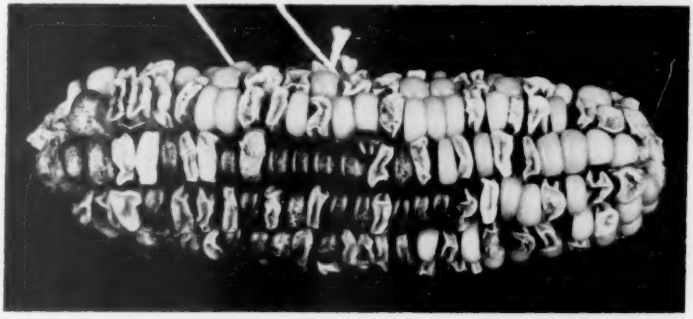

FIGURE 9.—Ear from the cross *a^m-Sh/a-sh, Dt Dt, p* × *a-sh, Dt Dt* showing a large sector segregating mutant *a^s-Sh* vs. parental *a^s-sh* types and a small sector segregating parental *a^m-Sh* vs. *a^s-sh*.

mutants, which have acquired the ability to perform some degree of *A* function in producing anthocyanin but which have lost the property of being able to mutate to a higher level of *A* action though not to lower forms; (2) unstable phenotypic mutants, which have acquired anthocyanin-producing properties and have retained the mutability of the parent allele; (3) changes in state of mutability. These mutants are phenotypically like the parent allele in that they are unable to produce anthocyanin but have either lost or undergone a change in mutability. It should be emphasized at this point that all mutational change considered here is dependent on the presence of *Dt*.

The first group of mutants includes stable anthocyanin-producing alleles which range in phenotypic level from the strongest known allele, A^r (full purple plant, full colored seed, red pericarp), through a continuous series of levels of pigmentation characterized by A^{rh} (full purple plant, full colored seed, red-brown pericarp), A^{br} (medium plant and seed color, recessive brown pericarp), down to A^{lt}, which has reddish brown plant color, dilute seed color, and recessive brown pericarp. These four classes constitute an arbitrary classification of what appears to be a continuous series of alleles.

The stability of these mutants (with respect to their ability to mutate further

to dominant alleles) can be established by direct observation only in the case of the medium and light color alleles A^{br} and A^{lt} since with these alleles if the locus still retained the a^m type of mutability one could expect to see medium or light colored seeds with dots of darker color which represent further mutations to dark color alleles. Such observations are impossible with the darker A^{rb} and A^r alleles since they already have a threshold or maximum pigmentation in the aleurone cells. However, it is possible to reduce their expression below this threshold by placing them in one dose in a heterozygote with a^b, an allele that partially suppresses the action of most A alleles (probably by competing for a substrate that is essential for anthocyanin production). The cross $a^b\ a^b \times A^r\ A^r$ yields seed with an endosperm of the constitution $a^b\ a^b\ A^r$. These seeds are considerably lighter in color than corresponding $A^r\ A^r\ A^r$ seeds. One then expects to find darker dots on an otherwise dilute seed if the new mutant A^r cases still retain the mutability of the a^m allele. Such was not the case in this group of alleles.

The second or mutable phenotypic group includes at present only two representatives, A^{br} mutable and A^{lt} mutable, hereafter designated $A^{br\ m}$ and $A^{lt\ m}$, respectively. The $A^{lt\ m}$ allele resembles the other A^{lt} alleles in most respects, giving a dilute aleurone, red-brown plant color and recessive brown pericarp. However, superimposed on this phenotype are many dots or sectors of darker tissue representing mutations to darker alleles. In addition to dots of darker color, seeds of the proper genotype ($a^s\ a^s\ A^{lt\ m}$) have frequent dot-like colorless sectors indicating mutation of $A^{lt\ m}$ to a as well as to A. One of these colorless dots which was quite large had within its borders a single dark A-like dot, suggesting that $A^{lt\ m}$ mutates to a mutable form of a which in turn can mutate to A. This fact was later confirmed by obtaining germinal cases of a from $A^{lt\ m}$.

The third class consisting of the cases of change in state of mutability, have, in the absence of *Dt*, the same phenotype as a^m. When *Dt* is present, however, they show distinct differences in mutability. With regard to these differences they can be classified into two types: (1) those capable of mutating to A but at a lower frequency as shown by the dots they produce (designated a^{m-}), and (2) those which are stable and fail to give dots in the presence of *Dt* (designated a^s). The first of these two includes alleles of several levels with regard to dotting, the highest of which has only one tenth the dotting ability of a^m and the lowest of which approaches the level of the a allele.

The foregoing description of mutant types and their mutational potentialities are summarized in the diagram of mutational sequences resulting from a^m (Figure 10). Not all the possibilities are listed, only those that are known to occur.

Frequency of Mutation: The mutants that have been described were collected by various methods from a number of sources with no attempt to determine their frequency. Separate experiments were conducted for this purpose. Ears from seven $a^m\ a^m$, *Dt Dt* cultures that had been pollinated by $a^s\ a^s$, *Dt Dt* were examined for whole-seed cases of the various mutant types. Care was taken to count grouped cases of the same type as single cases, as they were probably of a single mutant sector and would therefore distort the frequency if counted indi-

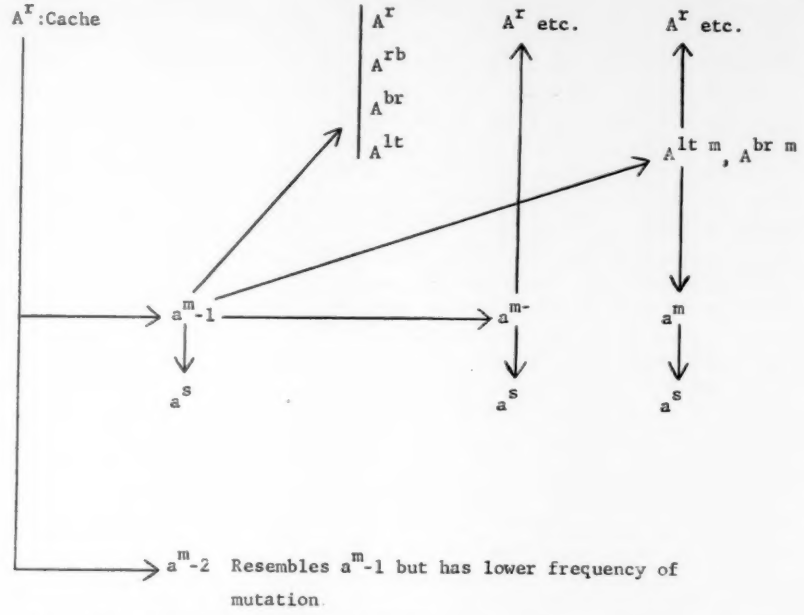

FIGURE 10.—Mutational sequence from *A:Cache*.

vidually. All the colored cases except A^{lt} were listed as A because there was no way to separate the A^r, A^{rb}, and A^{br} phenotype in the aleurone tissue of single-seed cases. The data are presented in Table 2. The a^s cases were not tested because they were growing in a part of the field destroyed by high water.

From this sample 48 A, 13 A^{lt}, and six a^{m-} cases received a test. Of these 21 A, four A^{lt}, and two a^{m-} cases transmitted the mutant type and therefore were good corresponding mutants which had arisen from a change that occurred early enough, or in the proper nuclei, to be included in both the endosperm and the embryo. Their frequency was 13, 3, and 2×10^{-4}, respectively. The remaining

TABLE 2

Frequency of mutant types among 15,450 seeds from a^m a^m. Dt Dt

	A	A^{lt}	$A^{lt\ m}$	a^{m-}	a^s
Total mutant phenotype	57	13	0	7	17
Number tested	48	13	0	6	0
Mutant embryo	21	4	0	2	..
Rate per 10,000	13	3	0	2	..
Nonmutant embryo	27	9	0	4	..
Rate per 10,000	18	6	0	3	..

27 *A*, nine A^{1t}, and four a^{m-} cases were not mutants but produced normal a^m a^s, *Dt Dt* plants. These were apparently cases in which the mutation had occurred at or after the initial division of the megaspore that produces the two nuclei, one of which through subsequent divisions contributes one nucleus to the embryo and one to the endosperm, while the other contributes one nucleus to the endosperm only. If a mutation occurs in the latter it can affect only the endosperm, while if it occurs in the former it can affect either or both. These cases that are not confirmed, that is, the mutant seeds which grew into nonmutant plants can also be used as a measure of mutation rate. They represent mutations occurring one division later, in those nuclei contributed to the endosperm only. The rate of mutation at this stage is 27 *A*, nine A^{1t}, and four a^{m-} from 15,450 chances or 18, 6, and 3×10^{-4}, respectively.

The many sizes of dots on a^m seeds suggest that a^m mutates at practically all stages of development. An accurate measure of the frequency of mutation to the *A* types, at a particular stage of development, can be obtained by counting the number of dots of a given size on a^m a^s a^s, *Dt Dt dt* seeds (homozygous *Dt* seeds from the above cross could not be used because they produced too many dots for counting). The population tested in such an experiment is the number of aleurone cells per seed, divided by the number of cells in the dots of the size selected for count. A count of two-cell dots was made of the top surface of 30 seeds from an ear from the cross a^s a^s, *Dt Dt* $\times$ a^m a^m, *dt dt*. They yielded a total of 808 cases with an average of 27 dots per 60,000-cell seed surface. The mutation rate then would be 27 in 30,000 or 9×10^{-4}.

Seeds of this same genotype can also be examined for half-seed sectors which represent mutations occurring when the chromatids of the primary endosperm nucleus are effectively separate. Each seed observed represents two chances for a mutation (expressed as a half-seed sector) to occur, hence the examination of 10,350 seeds represents a population of 20,700 chances. Five such sectors were found in this examination giving a mutation rate of 5/20,700 or 2.5×10^{-4}.

Similar examinations were made of a^m a^s a^s, *Dt Dt Dt* seeds from the cross of a^s a^s, *Dt Dt* $\times$ a^m a^m, *Dt Dt*. This is like the first cross described above except that male and female parents were reversed. Whole-seed mutants with corresponding mutant embryos cannot be counted in this case because a single tassel sector could produce many such mutant seeds (Figure 6). However, mutant seeds with nonmutant embryos avoid this problem and permit a measure of the frequency of mutation occurring at a stage between that in which the chromatids become effectively separate for the second microspore division and when they become separate again in the triple fusion nucleus. Among 5,870 seeds examined, 62 whole-seed mutants were found of which ten were mutant with nonmutant embryos thus giving a frequency of 10/5,870 or 17×10^{-4}.

These same seeds were examined for half-seed sectors like those previously described (except in this case there were three doses of *Dt*). The count was 64 among 5,870 seeds or 64/11,740 or 54×10^{-4}.

A summary of the data from each stage can be seen in Table 3. The right hand

TABLE 3

Summary of mutation frequencies (a^m *to* A) *at various stages of development*

Stage	Mutant expressed	Population	Mutants	Per 10^4	Doses of a^m and *Dt*	Corrected to 1 dose a^m and *Dt* per 10^4
Female gamete	Corresp. seed	15,450	25	16	2.2	2.3
Female endo. nucl.	Noncorresp. seed	15,450	36	24	2.2*	5.8
Last cell division	Two-cell dot	30,000	27	9	1.2	1.7
Prim. endo. nucl.	Half-seed sector	20,700	5	2.5	1.2	1.1
Male endo. nucl.	Noncorresp. seed	5,870	10	17	1.1	17.0
Prim. endo. nucl.	Half-seed sector	11,740	64	54	1.3	3.4

* The two *Dt* factors are in separate proendosperm nuclei hence the correction is on a linear not an exponential scale.

column lists the frequency after correction to one dose of a^m and one dose of *Dt*. Since changes in dose of a^m result in a linear change in dot frequency those cultures with two doses of a^m (line 1 and 2 of table) were corrected for this by dividing by two. Similarly, since an increase in dosage of *Dt* results in an exponential increase in dot frequency these results were corrected by taking the square or the cube root of the frequencies from two- or three-dose cultures, respectively. It should be pointed out that this manipulation of dot frequencies results in reduced differences between classes.

Even when one takes into account the above distortion, the differences are not great considering the other factors which could influence the frequency of dots. Such things as (1) length of stage during which a mutant must occur to be expressed in the manner observed, (2) differences in basic genotypes between cultures, and (3) differences in suitability of stage for mutational events all add a certain unmeasured amount of variability to the data. We are permitted to draw valid conclusions from this data with its several variables only because in comparison with mutation studies at other loci and with another allele of the *A* locus (*a*), the differences appear to be negligible. The *a* allele (and many other genes) mutates, with rare exception, at a particular time while a^m mutates at all stages. One can, therefore, conclude that, except for the effect of *Dt*, the time of mutation is more dependent on the allele involved than on the particular cellular environment.

DISCUSSION

The a^m allele and its associated modifiers present a unique combination whose analysis bears on several genetic problems including (1) mutational behavior (2) allelic variability and (3) genetic modification of mutability.

In previous studies of spontaneous mutation (usually measured as mutation to the recessive because these are the most frequent) rates have varied a great deal, depending on the definition of mutation, the gene involved, and on the genetic modifiers in the stocks involved. Frequencies ranging from zero to 18×10^{-4} have been reported in maize (STADLER 1939, 1949). These experiments and those

with other higher organisms generally have dealt with mutations that could be expressed in terms of frequency per gamete or per generation while a whole class of heritable changes that occur in the somatic cells and that are variously described as due to unstable genes, viral infections, chromosomal aberrations, etc., have been neglected. This was true, that is, until the amazing analysis of the mutator systems in maize by McCLINTOCK (1950, 1951). With our present constructs of hereditary material it is necessary to postulate gene mutation as the ultimate source of genetic variability, but modern researches have systematically reduced the reservoir of gene mutations by eliminating gross aberrations, deficiencies and then rearrangements within compound loci (LAUGHNAN 1949; STADLER and NUFFER 1953; STADLER and EMMERLING 1954, 1956). Mutation rate of a particular locus (*A*) in maize was thus reduced from 7.31×10^{-4} to 0.58×10^{-4} (LAUGHNAN 1952). One can follow this sort of reasoning to final elimination of all gametic mutants if one accepts LAUGHNAN's (1955) hypothesis that even the noncrossover mutants at the β locus may be due to intrastrand exchange. The so-called unstable loci, on the other hand, produce large numbers of mutant types which fit all the criteria of a change of the gene from one form to another. Furthermore, they produce dominant and intermediate mutants. something which normal genes rarely do.

When *Dt* is present the mtuable allele of the A_1 locus (a^m) described in this report produces a pattern of variegation comparable to that of other mutable loci in other species (DEMEREC 1935). McCLINTOCK (1950) offers convincing proof that the *a–Dt* system is similar to the *Ac–Ds* system which can be equated to others (BARCLAY and BRINK 1954; NUFFER 1955a). Yet when the mutation frequency of a^m in gametes (not to be confused with frequency of mutant gametes since a single mutant sector could produce a high percentage of mutant gametes) is measured, the rate 1.6×10^{-4} is not out of line with the previously reported rate of less than 1 to 18×10^{-4} reported for the *R* locus, (STADLER 1948) nor is the mutation rate in somatic cells when considered on the basis of cell generation ($1.1–14 \times 10^{-4}$) significantly higher than the gametic rate. The a^m allele, and perhaps many unstable genes, therefore, represent not highly mutable genes but genes which mutate at all stages of development.

One can argue that the previous reports of mutation frequencies for *R*, *C*, *Pr*, *Sh*, *Y*, etc., were per plant generation and for this reason were not comparable, but this is not entirely so. The mutants reported in most instances were single whole-seed cases produced on a nonmutant ear and were mutant in both endosperm and embryo. It was stated by STADLER (1946) that cases where the endosperm is mutant and the embryo nonmutant or *vice versa* were rare. There was no mention of large groups or sectors of mutant seeds which should appear if mutation had occurred at an early stage of plant development. The mutational event must have occurred at or near meiosis. If mutation occurred at an equal frequency at any other time, sizeable sectors of mutant tissue or noncorresponding endosperm-embryo cases should have been seen in frequencies comparable to those found for variegated genes. Quite likely, therefore, the mutations which

occur as single gametes, such as the type that have usually been considered typical of spontaneous mutation in general, are actually peculiar to and possibly a direct result of the meiotic process.

When one looks for possible sources of genetic variability one is struck by the way in which the mutable allele described in this report can provide it. The presence of *Dt* in a certain variety of corn may cause its A^r allele which is otherwise stable to mutate to a^m, which is highly mutable in the presence of *Dt* but stable otherwise. In those progenies still carrying *Dt*, the a^m mutates to produce many mutants of various allelic types which also may become stable (in those segregants lacking *Dt*) or continue to mutate (in those with *Dt*). Therefore, in a culture which is segregating for *Dt* a single A^r allele may produce many new alleles, each of which may be either stabilized to form a new unit for selection or may continue to be a source of further variability, depending on the absence or presence of *Dt* in the plants carrying the new allele.

Certainly such systems of control of mutation are widespread enough (NUFFER 1955b) and divergent enough in this action (MCCLINTOCK 1956) that a major share of genetic variability in maize could be attributed to this cause. Furthermore, these systems are not recent in origin but have been active in maize since prehistoric times as is shown by their wide distribution and the fact that one of them (the variegated pericarp system) is found in sorghum which is a distant relative of corn (G. F. SPRAGUE, personal communication).

It should be pointed out that the prevailing hypothesis for the mechanism of mutator systems, namely that of a system of controlling elements which suppress or release from suppression the locus involved (MCCLINTOCK 1951), also removes the mutable loci from the category of gene mutations (if defined as changes of the gene from one form to another). However, regardless of the mechanism involved the mutable loci do produce a wide variety of alleles which satisfy all the criteria for mutants and which provide an unlimited source of hereditary variability.

SUMMARY

A mutable allele of A_1 which mutates to several new allelic levels has been found. In the presence of *Dt* it mutates at all stages of development of the plant giving a high total mutation rate but a moderate rate when measured on a cell generation basis.

The mutational behavior of this allele was found to be quite different from the previously reported *Dt*-responding alleles and from other loci which have had extensive mutation trials.

The differences observed may be interpreted to indicate that previous studies of spontaneous mutation have dealt with phenomena which are peculiar to and possibly the result of the meiotic process.

The comparisons made suggest that the *a–Dt* system and other similar mutator systems may provide a large portion of the genetic variability found in corn.

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A GAMETOPHYTE FACTOR ON CHROMOSOME 5 OF CORN[1]

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A REVIEW of the early studies of the gametophyte factor (*ga*) on chromosome 4 of corn was presented by EMERSON (1934), when he published his data and discussion on this interesting pollen character. In certain female stylar tissue, the *ga* pollen that effects fertilization is reduced from the expected 50 percent to a mere 0–4 percent if it is competing with pollen carrying the dominant allele.

More recently SCHWARTZ (1950) suggested that a third allele (Ga^s) is at the *ga* locus of chromosome 4. NELSON (1952) studied this multiple-allelic series and reports that the reaction between the diploid style and the *ga* haploid pollen-tube, when competing with pollen tubes carrying a dominant allele, may be relative rather than absolute. He feels that environment may be one of the factors that serves to influence the progress of the competing pollen tubes down the stylar tissue.

BURNHAM (1936) found that chromosome 5 in certain corn strains showed differential fertilization. Since this publication, his students have obtained data to show that other chromosomes carry *ga* factors. These data have been presented in the Maize News Letter as their studies progressed.

Gametophyte factors such as those on chromosomes 4 and 5 of corn have certain characteristics that make their transmission difficult to follow. Even the detection of a *ga* factor requires that it be linked with a factor with a visible effect.

The presence of a *ga* factor in the stock of translocation #5614, which has breaks near the centromeres of chromosomes 5 and 9, was detected in 1954. Later studies of this translocation demonstrated that the *ga* factor was on chromosome 5.

MATERIALS AND OBSERVATIONS

Transmission of translocation #5614 was found to be abnormal by E. G. ANDERSON during his preparation of seed stocks of the many translocations he has isolated.

The first small progeny grown by the writer contained two homozygous translocation plants. Both plants were also homozygous for a necrotic (*nc nc*)

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factor that produces necrotic areas on the first leaves of seedlings. These areas usually expand and the plants rarely grow to maturity.

Linkage between *nc* and the translocation is close, so most plants homozygous for the translocation were lost during attempts to raise them. A second cause for a low percentage of homozygous translocations in selfed progenies of heterozygous T #5614 became apparent when it was discovered that most plants were also heterozygous *Ga ga* and that *ga* was closely linked to the translocation. The close linkage between *ga* and the translocation reduces the pollen transmission of this translocation.

The transmission studies of this *ga* factor on chromosome 5 were aided by its close linkage in T #5614 to *Wx* on chromosome 9 (*wx wx wx* endosperms and *wx* pollen grains stain reddish-brown with iodine, rather than the normal blue). Seven semisterile plants in the original small selfed progeny were selfed and outcrossed onto *wx wx* egg parents. The resultant ears segregated *Wx* and *wx* kernels and were used to measure *Wx* transmission. The egg transmission of *Wx* for six of these plants was normal, but the pollen transmission was only 15 percent *wx* instead of the expected 50 percent. The seventh semisterile *Wx wx* plant differed from the others in that it had normal transmission of *Wx* in both eggs and pollen. Later tests, however, showed that this plant was also *Ga ga* in constitution. This observation gave impetus to a study of *ga* transmission. It soon became evident that a specific female tester type is required to bring about distortion of transmission ratios of *Ga* and *ga* pollen and that *ga* transmission behaves as a two-factor character.

To work with these two factor pairs requires two strain types. One must produce both *ga* and *Ga* pollen; the other must have the ability to select the *Ga* pollen type preferentially so that it effects fertilization above the expected ratio.

Data to illustrate the independent nature of these two factors that together distort pollen transmission were provided by semisterile plants producing *Ga Wx*, *Ga wx*, *ga Wx*, and *ga wx* pollen. Table 1 gives data from tests that provided paired ears, one selfed and the other an outcross, with the pollen for both coming from the same plant. Ear pairs that showed only normal pollen transmission of *wx* were omitted from the table; the others are grouped according to their transmission pattern. It is clear from these tests that the aberrant transmission of linked *Wx–ga* through the pollen is due to some characteristic of the female parent distinct from *Ga*.

TABLE 1

Transmission of wx *pollen from semisterile plants (T #5614) heterozygous for* Wx wx *and* Ga ga

Outcrosses				Selfs		
Plants no.	Seeds no.	Freq. of *wx* Obs.	Exp.	Seeds no.	Freq. of *wx* Obs.	Exp.
249	40769	.94	.50	26337	.45	.25
127	18958	.93	.50	12990	.24	.25
25	5158	.45	.50	2118	.45	.25
1	11	.102	.50			

Tests of the type shown in Table 1 were made to measure the transmission of factors linked to each other and to *ga* in segregating *Ga ga* plants. These tests included, in addition to *wx* and *nc*, brittle (*bt*), an endosperm factor causing seeds to be much shrunken and translucent; red aleurone (*pr*), which gives red-colored seeds in the presence of other genes necessary for aleurone color; virescent seedling-2 (v_2), giving very light yellow seedlings; and shrunken endosperm (*sh*), in which the endosperm collapses during drying, giving prominent indentations at the crown or at the side of the seed.

Those plants that gave abnormal ratios for pollen transmission frequently had been tested for egg transmission, and the data for samples from these female transmission tests are given in Table 2.

The independent nature of the two factors which together distort the trans-

TABLE 2

Transmission of linked factors through eggs of semisterile plants (T #5614) heterozygous for Ga ga

Character	Ears no.	Individuals no.	Frequency Obs.	Exp.
Semisterile	5	589	.49	.50
wx	50	5542	.50	.50
sh	19	2515	.26	.25
nc	9	511	.22	.25
bt	6	655	.24	.25
pr	34	2753	.50	.50

TABLE 3

Transmission of linked factors through pollen of semisterile plants (T #5614) heterozygous for Ga ga

Character	Outcrosses Ears no.	Indivs. no.	Frequency Obs.	Exp.	Selfs Ears no.	Indivs. no.	Frequency Obs.	Exp.
Semisterile	12	1869	.11	.50	..	....	..	..
	5	175	.52	.50	..	....	..	..
	1	11	.90	.50	..	....	..	..
sh	7	1022	.40	.25	7	933	.39	.25
	5	1070	.09	.25	5	727	.10	.25
	5	882	.41	.25	5	558	.23	.25
nc	2	97	.04	.25	41	3118	.03	.25
	2	115	.25	.25	5	250	.02	.25
bt	2	297	.01	.25	129	14696	.02	.25
					39	4973	.25	.25
pr	29	3149	.81	.50	39	2602	.41	.25
	19	1452	.48	.50	19	1746	.26	.25

mission of pollen from *Ga ga* plants is further illustrated by the data presented in Table 3. This table gives ratios for other factors linked to *ga* and for each there are ratios similar to those given for *wx* in Table 1. The data given in Tables 1–3 may be summarized as follows: (1) There is normal transmission of the *ga* factor through eggs. (2) Through the pollen, there is abnormal transmission of the *ga* factor in competition with *Ga* only if the female tester plant carries a factor that prevents all or part of the *ga* pollen from effecting fertilization of the eggs.

It was frequently observed that between the normal and the most abnormal pollen transmission ratios for factors on chromosome 5 there was an appreciable array of intermediate ratios. This array suggested the possibility of more than one *Ga* factor on chromosome 5. The spread in ratio values for selfed plants segregating *nc* and *bt* is shown graphically in Figure 1. Ratios from semisterile plants show a prominent peak in the low-ratio part of the two curves, because low-ratio ears were the first to attract attention and continued to be numerous in these studies. As later progenies from these first ears were grown, the part of the two curves around the normal 25 percent ratios developed. This part of the curves for semisterile plants and the complete curves for plants without the

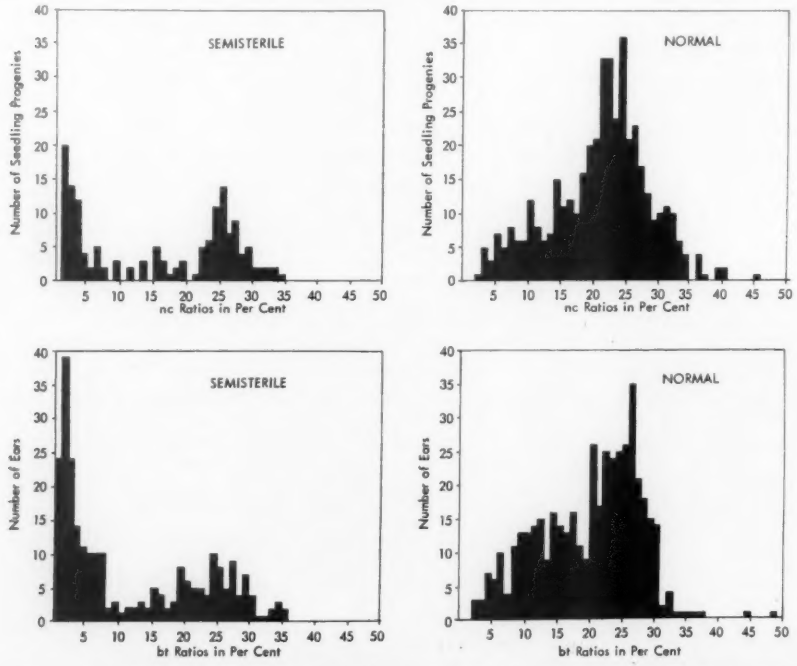

FIGURE 1.—Distributions of ratios for brittle endosperm *(bt)* and necrotic seedling *(nc)* among ears from self-pollinated semisterile and normal plants.

translocation have no prominent peaks except the ones at the 25 percent transmission points. The data suggest nothing to support a multiple array of *ga* loci on chromosome 5. One *Ga* locus with factors modifying the eliminating character of the female parent seems sufficient to produce the variable transmission ratios observed.

BURNHAM (1936) recognized the possibility that "*ga* gametes are not completely eliminated in the pollen." Tests were included in the present studies that confirmed incomplete elimination of *ga* gametes from the pollen. Markers linked with *ga*, when transmitted through the pollen and classified as presumptive crossovers, were found to include 16 to 50 percent noncrossovers that retained *ga*. There was therefore transmission to the eggs of a certain amount of *ga*-carrying pollen. Some of the data for the two types of plants found among those classified as crossovers are given in Table 4.

Since the crossover class for a factor linked to *ga* may be diluted by the transmission of an unpredictable percentage of *ga*-bearing pollen, the true crossover values for factors linked to *ga* are difficult to obtain. Transmission ratios for *Wx* are more readily followed than are those for some of the other factors linked to *ga* by T #5614. *Wx-Ga* recombination values were calculated and are given in Table 5 along with values for several of the factors linked to *nc* and to the translocation. Progeny tests of seedlings and plants from ears that gave no distortion

TABLE 4

Progenies from individual outcrossed ears of wx × Ga wx/ga Wx *showing the transmission of* Ga Wx *and* ga Wx *pollen*

Seeds no.	Noncrossovers *ga Wx* frequency	Crossovers *Ga Wx* frequency
68	.19	.08
316	.02	.01
151	.14	.12
151	.04	.06
108	.15	.09
316	.04	.07
205	.04	.01

TABLE 5

Linkage data for some of the factors used in this study

Genes X, Y	Phase	X Y	X y	x Y	x y	Total no.	Recombination percent
Wx Ga	RB	59	..	1178	..	1237	5
Wx semisterile	CB	139	17	4	135	295	7
Wx Nc	RS	119	55	52	1	227	14
Sh Nc	RS	164	76	66	4	310	24
Wx Bt	RS	195	99	97	2	393	14
Nc Pr	RS	292	130	135	10	567	26
Nc V_2	RS	161	69	57	8	295	35

in the pollen transmission rates were used in obtaining crossover values for factors linked to *nc* and the translocation. The *Nc-Pr* and *Nc-V_2* data were obtained from plants normal for chromosome 5.

The one value given in Table 5 for *Wx-Ga* recombination in semisterile plants, combined with the general observation that many factors linked with *ga* are almost completely eliminated, places this locus close to the translocation, to *nc*, and to *bt*.

DISCUSSION

Studies of the behavior of *ga* factors have progressed with each investigator's attempt to analyze their role in transmission disturbances.

The position of *Ga* on chromosome 5 has been approximately established. The segregation of *ga* and *Ga* in heterozygous plants is strictly Mendelian. The female recipient of pollen from a segregating *Ga ga* plant is responsible for the distorted ratios of pollen transmission. These facts seem well established.

Sometimes all plants in a progeny screen out effectively the transmission of *ga* pollen to the egg. Other progenies are variable in their ability to eliminate *ga* pollen. BURNHAM (1936) and NELSON (1952) suggest the possibility that variations in the environment may contribute to the variability in pollen transmission. The present studies support the view that environmental and other modifiers of this plant character cause variable pollen transmission of chromosome 5 and factors associated with it whenever *ga* and *Ga* are segregating.

The *ga* gene may be generally distributed among genetic stocks and among corn varieties. The proper female tester stocks are, however, necessary for its detection. One searches for a descriptive label for the property of certain female stocks that makes them effective testers for segregating *Ga* and *ga*. Tests indicate that this character is dominant, although this dominance may be subject to modification so that heterozygotes may be less effective in elimination of *ga* pollen. Tests show that this character is rarely if ever 100 percent effective in *ga* pollen elimination and probably is affected by modifying factors. Nothing that gives a clue to the map location of the responsible factor has been discovered beyond the fact that it seems to show no linkage with *ga*.

The cross-sterility of the Black Beauty pop variety of corn suggests that it is a female tester strain. If so, it will have eliminated heterozygous forms of *Ga ga* from its constitution. Black Beauty is self-fertile but partially or completely cross-sterile if it is pollinated by certain corn varieties.

It is hoped that these data accumulated during the past four years make a little more understandable the role of *ga* in pollen transmission of chromosome 5 and that conclusions drawn from them may have some bearing on the occasional erratic pollen transmission of other chromosomes carrying a *ga* factor.

These studies have introduced the problem of locating the factor that causes the preferential passage of *Ga* over *ga* pollen through the tissue of the recipient. Crosses to a translocation series were started last season with linkage determination in mind, but these studies are being discontinued.

SUMMARY

Aberrant pollen transmission of factors on chromosome 5 of corn occurs when pollen segregating for *Ga ga* grows on a certain type or certain types of female tissue. The *ga* pollen is partially or completely blocked from reaching an egg by a second factor acting in the female tissue. The strength of the blocking is modified by one or more modifying factors or by environment.

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LINKAGE STUDIES OF MUTANTS IN MAIZE WITH PIGMENT DEFICIENCIES IN ENDOSPERM AND SEEDLING[1]

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THIS paper will be concerned mainly with those pigment defective mutants of maize that have pale yellow or white endosperms and chalky-white albino or pale green seedlings. Most early workers with chlorophyll deficient mutants of maize report only on seedling phenotype and do not mention the color of the endosperm. The first report of albino mutants which are also deficient for carotenoids in the endosperm are those of LINDSTROM (1923), EYSTER (1924a,b, 1931) and MANGELSDORF (1923, 1926, 1930). The latter two workers were primarily concerned with studying viviparous mutants which in most instances also have white endosperms and albino seedlings. More recent reports of genetic work with these mutants have been made by EVERETT (1949), TULPULE (1954), ROBERTSON (1955) and SIDHU (1960).

There have been reports in the literature of two mutants that have white endosperm and pale green seedlings. One of these was a description of an unnamed mutant by ANDERSON (1924). The other mutant, albescent, was first described by PHIPPS (1929), and was placed on chromosome 2 by PERRY and SPRAGUE (1936). GRANER and ACCORSI (1949) compared the development of chloroplasts in this mutant with those of a white endosperm albino. There is some confusion in the symbols used for the albescent mutant. The symbol y_3 was used for the white endosperm condition and *al* for the seedling phenotype. Early, it was felt that two closely linked genes were involved. However, the work of PERRY and SPRAGUE (1936) would suggest that the reported crossovers are best explained by heterofertilization.

Recent work by KOSKI and SMITH (1951), SMITH, DURHAM and WURSTER (1959), ANDERSON and ROBERTSON (1960), and ANDERSON (unpublished) has suggested that the normal alleles of these mutants are concerned with carotenoid production and that chlorophyll is only secondarily involved. The present evidence suggests that yellow carotenoids are necessary for the protection of chlorophyll from oxidative photodestruction (ANDERSON and ROBERTSON 1960). The mutant seedlings, most of which are completely devoid of yellow carotenoids, accumulate chlorophyll if grown under dim light. However, the chlorophyll is destroyed as fast as it is formed under normal growing conditions where light is plentiful.

The promising results obtained from the biochemical studies with these

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mutants by the above workers, as well as the more recent genetic studies by SIDHU (1960) and ROBERTSON (1960) would indicate that these pigment deficient mutants will be subject to further biochemical and genetic studies in the near future. Therefore, it is of value to have precise linkage information available. ROBERTSON (1955) presented linkage data that was then available for the viviparous albino mutants. Since that time, additional linkage data have been obtained which make possible the placing of these genes with more precision. These new data will be presented as well as linkage data for the nonviviparous albino mutants.

MATERIALS AND METHODS

Description of mutants

Because mutants of divergent phenotypes will be discussed, it is advisable to group them for convenience of reference. ROBERTSON (1955) suggested that these mutants be classified into three groups: (1) those with pale yellow or white seeds and albino viviparous seedlings, (2) those which show little if any tendency to germinate prematurely but are otherwise similar to Class 1, and (3) those which are similar to Class 1 in having pale yellow or white seeds which do not germinate prematurely but differ in that they produce pale green seedlings.

Subsequent allele tests with pastel-4889 and pastel-8686, both of which belong to Group 3 and have never shown any tendency to be viviparous, have indicated that they are allelic to the Group 1 mutants vp_9 and w_3 respectively. Further allele tests have created additional difficulties for establishing a satisfactory system of classification of these mutants. Pastel-8549, and white-mutable, Group 3 mutants, have been shown to be allelic to y_1 (ROBERTSON and ANDERSON 1961), which is not a member of any of the three groups.

The results of these allele tests indicate that the classification suggested above is an artificial one, since it places allelic mutants in different groups. However, in spite of this, such a system of classification is useful for grouping mutants with similar phenotypes if it is understood that it is based solely on phenotype and in no way implies genotypic relationships. In order to incorporate the results of the allele tests, a further group will be added giving a total of four, as summarized in Table 1.

Different names and symbols have been used by various workers to designate these genes. No attempt will be made here to settle on a unifying system nomen-

TABLE 1

A summary of the phenotypes that characterize the groups of mutants discussed in this report

Group	Phenotype of endosperm	Phenotype of plant
1	white	albino-viviparous
2	white	albino
3	white	pale green (pastel)
4	white	green

clature. This cannot be done intelligently until more is known about the chemistry of these mutants and until their relationship to other pigment deficient mutants of corn is better understood.

The names and symbols used in this report will be those of the workers that first found and/or published on the particular gene under consideration. Where alleles are discussed, they will usually be designated by a name and/or symbol that implies their source (e.g., $w_{Everett\ No.\ 1}$, w_{Smith}, $w_{Sprague}$, and $w_{Brown\ No.\ 1}$, etc.) Symbols that contain a four number subscript (e.g., w_{6474}, w_{7748}) stand for genes that were found in stocks derived from seed exposed to the Bikini atom bomb and other sources of irradiation that were first grown at the California Institute of Technology in Pasadena. California.

Source of genes

The sources of the genes vp_2, vp_5, vp_9, *ps*, and w_3 are given by ROBERTSON (1955). Lemon-white-1, lw_2, lw_3, and lw_4 were obtained from DR. M. M. RHOADES and their origin is discussed by TULPULE (1954). The cl_1 mutant was obtained from DR. HERBERT L. EVERETT and its origin has been described by him (EVERETT 1949). Mutant w_{7748} and its allele, w_{8624}, were first found in stocks derived from irradiated seed grown at the California Institute of Technology. The origins of pas_{8549} and w_{mut} are given by ROBERTSON and ANDERSON (1961). Seed of the albescent mutant was obtained from DR. GEORGE F. SPRAGUE.

Linkage techniques

Extensive use has been made of gene marked translocations and translocations with B-type chromosomes in locating genes in these studies. ANDERSON (1952, 1956) and ROBERTSON (1955) have described the principles involved in the use of gene marked translocations and ROMAN and ULLSTRUP (1951) have described the use of translocations with B-type chromosomes in locating genes.

Because albino plants do not survive beyond the seedling stage, it is impossible to obtain the necessary double and triple homozygotes for testcrossing. This necessitated the use of the modified testcross as described by ROBERTSON (1955).

Genetic studies

Viviparous-2: Allele tests have revealed that a similar mutant, green mosaic, which first appeared in the stocks at the California Institute of Technology, was allelic to vp_2. Both of these mutants belong to Group 1, but green mosaic differs from vp_2 in that it is an unstable gene that regularly reverts to normal in both endosperm and seedling. Green mosaic seeds are white or pale yellow with spots of dark yellow tissue and the seedlings are albino with small streaks of normal green tissue. Very little is known about the nature of the mutable system in this mutant. The presence of mutable and stable white seeds and seedling on the same selfed ear indicates that an independent modifier is involved.

The data reported by ROBERTSON (1955) placed vp_2 in the short arm of chromosome 5 to the left of bm_1. The results of the three point tests recorded in Table 2 indicate that vp_2 is to the right of a_2. The *ps* mutant is another Group 1

TABLE 2

Summary of three point data from testcrosses involving vp_2

Genotype of F_1	Parental combinations		Recombinations: Region 1		Region 2		Region 1 and 2		Totals	Percent recombinations: Region 1	Region 2
$\frac{a_2\ vp_2\ +}{+\ +\ ps}$	167	171	3	2	1	2	1	0	347	1.7	1.2
$\frac{+\ +\ bm_1}{a_2\ vp_2\ +}$	41	36	1	2	5	2	0	0	87	3.4	8.0

albino mutant (see below). Table 3 summarizes the linkage data for vp_2 and its nearest neighbors. These data indicate the following linkage relationship: a_2–3.3–vp_2–0.9–*ps*–3.3 bm_1.

Viviparous-5: The following five alleles of this Group 1 mutant have been found: w_{s605} and w_{K40}, $w_{Mumm\ No.\ 1}$, $w_{Brawn\ No.\ 1}$ and $w_{Sprague\ No.\ 1}$. All of these alleles are also Group 1 mutants.

ROBERTSON (1955) placed this gene in the short arm of chromosome 1 to the left of *P*. The data from Table 4 permit the placing of this gene with some precision. It will be noted that the data included in this table were obtained from a self. This was made necessary because a series of unfortunate coincidences prevented obtaining the necessary testcrosses. Non-vp_5 seeds from selfed ears of plants of the genotype *T1-2c sr* +/+ + vp_5 were planted and the resulting plants classified for sterility and striate and then self pollinated. The ears from these F_2 plants were classified for vivipary and seedling tested to determine the striate constitution. With this information, it is possible to determine the genotype of the nonviviparous gametes that gave rise to the F_2 plants. Viviparous classes cannot be used because it is not possible to classify for striate when vp_5

TABLE 3

Summary of two point data from testcrosses involving vp_2

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{a_2\ +}{+\ vp_2}$	132	137	9	6	284	15	5.3
$\frac{+\ +}{a_2\ vp_2}$	219	206	3	6	434	9	2.1
					718	24	3.3
$\frac{+\ ps}{vp_2\ +}$	208	218	3	1	430	4	0.9
$\frac{+\ bm_1}{vp_2\ +}$	182	209	6	11	408	17	4.2

TABLE 4

Non-vp_5 *gametes functioning in self of* $\frac{\text{T1-2c sr +}}{+ \quad + \; vp_5}$

F_1 parents	Parental combinations *T sr* +	Recombinations: Region 1 + *sr* +	Region 2 + + +	Region 1 and 2 *T* + +	Totals	Percent recombinations: Region 1	Region 2
58-5638-75	135	2	2	0	139	1.4	1.4
58-5640-14	138	0	1	0	139	—	0.7
58-5642-18	101	0	1	0	102	—	1.0
58-5643-14	115	3	0	0	118	2.5	—
Totals	489	5	4	0	498	1.0	0.8

is present. It is thought that these results are very reliable since very little crossing over is observed in either regions 1 or 2. The data from this table indicate an order of *T1-2c–sr–*vp_5.

Table 5 gives linkage data for vp_5 with ts_2 and *P*. Backcross data reported by EMERSON (1939) and ANDERSON (1941) indicate that ts_2 is about one unit to the left of *P*. The data from Table 5 would indicate that the order of these two genes might be reversed. However, since the numbers are small, especially in the case of the linkage with *P*, the discrepancy here might be the result of sampling error. Assuming that ts_2 is the left most gene, the present data indicate the following linkage relationship for vp_5: *T1-2c*–1.0–*sr*–0.8–vp_5–23.4–ts_2. Since *T1-2c* has been placed by LONGLEY (1958) in the short arm of chromosome 1 at .77, this would mean that *sr* and vp_5 are proximal to this break point. It must be kept in mind that the presence of *T1-2c* in heterozygous condition in the F_1 may have reduced the true crossover value for the *T-sr* and *sr-*vp_5 regions.

Viviparous-9: Two alleles to this Group 1 mutant have been found: $Pastel_{4889}$, a Group 3 mutant; and $w_{Brown\ No.\ 2}$, a Group 1 mutant.

ROBERTSON (1955) indicated that this gene was in the long arm of chromosome 7 and that the most likely order was *T7-9a*—gl_1—vp_9. This order was based on two point tests and assumed the then reported position of L.29 for the break point of *T7-9a* in the long arm of chromosome 7. Since then three point tests have been made (Table 6) and a corrected estimate of the break point as 7 L.63 has been published (LONGLEY 1958). Using this additional information and the linkage data from Table 7, the linkage relationships for vp_9 can be summarized as follows:

TABLE 5

Summary of two point data from testcrosses involving vp_5

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{+ \; ts_2}{vp_5 \; +}$	122	107	40	30	299	70	23.4
$\frac{+ \; P}{vp_5 \; +}$	75	69	26	15	185	41	22.2

vp$_9$—10.9—*gl*$_1$—17.9—*T7-9a*. This would place *vp*$_9$ in the neighborhood of the centromere on chromosome 7.

Pink scutellum: Twelve alleles of *ps*, a Group 1 mutant, have been found. All of these also belong to Group 1 and were first isolated from stocks grown at the California Institute of Technology.

Based on the data that were then available, ROBERTSON (1955) tentatively suggested that *ps* was to the left of *pr*. Subsequent tests have confirmed this position and the present data from Table 8 definitely place *ps* to the left of *pr* and

TABLE 6

Summary of three point data from testcrosses involving vp$_9$

Genotype of F_1	Parental combinations		Recombinations Region 1		Recombinations Region 2		Region 1 and 2		Totals	Percent recombinations Region 1	Percent recombinations Region 2
$\frac{+\ gl_1\ +}{vp_9\ +\ T7\text{-}9a}$	52	58	13	8	10	7	4	1	153	17.0	14.4
$\frac{+\ +\ T7\text{-}9a}{vp_9\ gl_1\ +}$	32	41	5	6	8	14	0	3	109	12.9	22.9
Totals	84	99	18	14	18	21	4	4	262	15.3	17.9

TABLE 7

Summary of two point data from testcrosses involving vp$_9$

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{+\ gl_1}{vp_9\ +}$	257	299	40	26	622	66	10.6
$\frac{+\ +}{vp_9\ gl_1}$	40	55	5	9	109	14	12.8
Totals					731	80	10.9

TABLE 8

Testcross progenies of $\frac{+\ \text{bm}_1\ \text{pr}\ +}{+\ \text{bm}_1\ \text{pr}\ +} \times \frac{+\ \text{bm}_1\ \text{pr}\ +}{\text{ps}\ +\ +\ \text{T5-9a}}$

F_1 parents	Parental combinations		Reg. 1		Reg. 2		Reg. 3		Reg. 1&2		Reg. 1&3		Reg. 2&3		Reg. 1, 2&3		Totals
58-5291-7	16	26	1	0	10	10	10	14	1	0	0	0	0	0	0	0	88
58-5292-8	26	25	1	0	3	8	15	6	0	1	0	0	0	0	0	0	85
Totals	93		2		31		45		2		0		0		0		173
Percent Recombination			1.2		17.9		26.0		1.2								

in the neighborhood of bm_1. Although the data from Table 8 will permit the placement of *ps* with respect to *pr*, they are not so helpful in placing the gene with respect to bm_1. However, linkage results given in Tables 2 and 3 would suggest that *ps* is distal to bm_1 and proximal to a_2 and vp_2. Using the available information, the following linkage relationships are indicated: a_2—3.3—vp_2—0.9—*ps*—2.4—bm_1.

White-3: Two alleles of this Group 1 mutant have been found: $w_{Everett\ No.\ 1}$, a Group 1 mutant; and $Pastel_{sesse}$, a Group 3 mutant.

Because of linkage found with *T2-4c*, ROBERTSON (1955) had placed this gene on chromosome 2. The data from three point tests involving v_4 are given in Table 9. All of these are consistent in indicating that w_3 is to the right of v_4 in the long arm of chromosome 2. Table 10 (first cross) summarizes the linkage information available for v_4 and w_3 for those crosses where translocations were

TABLE 9

Summary of three point data from testcrosses involving w_3

Genotype of F_1	Parental combinations		Recombinations Region 1		Recombinations Region 2		Region 1 and 2		Totals	Percent recombinations Region 1	Percent recombinations Region 2
$\frac{B\ v_4\ +}{+\ +\ w_3}$	63*	92	54	26*	17*	41	7	4*	304	29.9	22.7
$\frac{ts_1\ v\ +}{+\ +\ w_3}$	0*	37	10	4*	1*	16	2	2	72	25.0	29.2
$\frac{+\ +\ T2\text{-}4c}{v_4\ w_3\ +}$	77	80	17	31	1	1	0	0	207	23.2	1.0
$\frac{+\ +\ T2\text{-}9d}{v_4\ w_3\ +}$	96	65	15	18	0	1	0	0	195	16.9	0.5

* The numbers in these classes are low because of low viability of v_4 plants.

TABLE 10

Summary of two point data from testcrosses involving w_3

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{v_4\ +}{+\ w_3}$	93*	193	24*	66	376	90	23.9
$\frac{+\ T2\text{-}4c}{w_3\ +}$	283	288	4	4	579	8	1.4
$\frac{+\ Ch}{w_3\ +}$	91	83	51	38	263	89	33.8

* The numbers in these classes are low because of low viability of v_4 plants.

not involved. These data indicate 23.9 percent recombination between these two loci. The three point tests with *T2-4c* and *T2-9d* indicate that these translocations are probably distal to w_3. LONGLEY (1958) has indicated the break points in chromosome 2 to be L.81 for *T2-4c* and L.83 for *T2-9d*. The similar linkage values with w_3 observed for these two translocations agree with the cytological observation that their break points are very close together. A point test with chocolate (Table 10, third cross) gave 33.8 percent recombination with w_3. Since *Ch* is the most distal gene indicated by RHOADES (1954) on the long arm of chromosome 2, it is most likely that w_3 is proximal to it. The data from Tables 9 and 10 indicate the following linkage relationship for w_3: v_4—23.9—w_3—1.2—"*T*"—32.6 *Ch*. Because the break points of *T2-4c* and *T2-9d* are so close, "*T*" in this summary is used to stand for both of them and the crossover value of 1.2 was obtained by combining the linkage data for the two translocations.

Lemon-white-1: TULPULE (1954) had located this Group 2 mutant on the long arm of chromosome 1 by the use of *TB-1a*. *White*$_{6474}$, a Group 2 mutant, which had also been placed on chromosome 1 by linkage with *T1-4a*, proved to be allelic to lw_1.

The limited three point data from Table 11 suggest that lw_1 is proximal to the break point of *T1-4a* which has been placed at 1L .51 (LONGLEY 1958). Table 12,

TABLE 11

Summary of three point data from a testcross involving lw_1

Genotype of F_1	Parental combinations		Recombinations Region 1		Recombinations Region 2		Region 1 and 2		Totals	Percent recombinations Region 1	Percent recombinations Region 2
$\frac{P + T1\text{-}4a}{+ lw_1 +}$	19	14	11	16	7	9	6	7	89	44.9	32.6

TABLE 12

Summary of two point data from testcrosses involving lw_1

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{+ T1\text{-}4a}{lw_1 +}$	99	67	36	32	234	68	29.1
$\frac{+ +}{lw_1 T1\text{-}4a}$	69	86	20	25	200	45	22.5
					434	113	26.0
$\frac{Kn +}{+ lw_1}$	234	292	0	6	532	6	1.1
$\frac{+ bm_2}{lw_1 +}$	58	51	16	18	143	34	23.8

(first and second crosses) summarizes the linkage tests with *T1-4a* and indicates that lw_1 is 26.0 crossover units from the break point in chromosome 1. Linkage tests with *Kn* indicate very close linkage (Table 12, third cross). The figure of 1.1 percent recombination is probably a maximum value. It will be noticed that the crossovers are all in the + + class. Since any seeds resulting from contamination would fall in this class, these may not represent actual crossovers. So far no crossovers of the *Kn* lw_1 class have been observed. A recombination value with bm_2 of 23.8 percent has been found (Table 11, fourth cross). The genetic studies with lw_1 indicate the following linkage relationships: *Kn*–(1.1)–lw_1–23.8–bm_2. At present, it is impossible to place lw_1 to the left or right of *Kn*.

Lemon-white-2: Using F_2 data TULPULE (1954) had placed lw_2, a Group 2 mutant, on chromosome 5. *White* $_{7752}$, which had been placed on chromosome 5 by linkage with *T5-9c*, and the unplaced genes $w_{Everett\ No.\ 3}$, $w_{Smith\ No.\ 1}$, $w_{Brunson}$, w_{Chase} have proven to be allelic to lw_2. All these alleles are Group 2 mutants.

The results of three point tests with *pr* given in Table 13 (first and second crosses), while inconclusive, would suggest that lw_2 is to the right of this gene. The summary of all linkage data with *pr* (Table 14, first cross) indicate 2.2 percent crossing over between this locus and lw_2. The three point tests with *ps*

TABLE 13

Summary of three point data from testcrosses involving lw_2

Genotype of F_1	Parental combinations		Recombinations Region 1		Region 2		Region 1 and 2		Totals	Percent recombinations Region 1	Region 2
$\frac{+\ pr\ +}{ps\ +\ lw_2}$	75	72	11	14	0	1	2	0	175	15.4	1.7
$\frac{bm_1\ pr\ +}{+\ +\ lw_2}$	71	78	19	26	1	3	0	1	199	23.1	2.5
$\frac{+\ +\ T5\text{-}9a}{ps\ lw_2\ +}$	71	46	12	41	8	22	2	6	208	29.3	18.3
$\frac{+\ T5\text{-}9d\ +}{ps\ +\ lw_2}$	67	99	0	0	17	17	0	1	201	0.5	17.4

TABLE 14

Summary of two point data from testcrosses involving lw_2

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{pr\ +}{+\ lw_2}$	227	225	4	6	462	10	2.2
$\frac{+\ gl_8}{lw_2\ +}$	207	202	0	0	409	0	0

and translocations *T5-9a* and *T5-9d* (Table 13, third and fourth crosses) indicate that lw_2 is proximal to the break point of the former translocation at 5L.69 (LONGLEY 1958) 18.3 units and distal to the break point of the latter at 5L.14 (LONGLEY 1958) 17.4 units. Linkage tests with gl_8 (Table 14, second cross) have revealed no crossing over in 409 plants tested.

The following is a summary of the available linkage information on lw_2: *T5-9d*—15.2—*pr*—2.2—lw_2gl_8—18.3—*T5-9a*.

Lemon-white-3 and Lemon-white-4: These duplicate genes are Group 2 mutants and were first described by TULPULE (1954). On the basis of F_2 data, TULPULE (1954) placed lw_3 on chromosome 5 to the right of bt_1 in the long arm and lw_4 on chromosome 4, 13 units to the right of su_1 and 33 units to the left of gl_1. No further linkage data for these mutants are available at present.

Chlorophyll-1: This mutant was first placed to chromosome 3 by means of linkage with *T3-9c*. Allele tests have turned up two Group 1 alleles, w_{7716} and $w_{Pioneer}$.

Linkage data from Table 15 indicate that cl_1 is located between ra_2 and lg_2 and to the left of the break point of *T3-9c* which LONGLEY (1958) placed at 3L.09. Table 16 (first and second crosses) summarizes all available linked information with this translocation and indicates 4.6 percent crossing between cl_1 and the break point in chromosome 3. Table 16 (third–fifth crosses) gives the results of two point tests involving ra_2 and lg_2. Since ANDERSON and RANDOLPH (1945) indicate lg_2 is well out in the long arm of chromosome 3 and since cl_1 is very close to the break point of *T3-9c* at 3L.09, it is very likely that these three genes lie in the order given. The results of linkage studies with cl_1 indicate the following map: ra_2–11.9–cl_1–4.6–*T3-9c*–22.7–lg_2. Because of the small numbers of plants tested in the three point test with *T3-9c*, the placement of cl_1 with respect to this translocation is still rather tenuous. The cl_1 locus must be very close to the centromere in chromosome 3.

White$_{7748}$: This is a new Group 2 mutant that has not been described before. It first appeared in stocks derived from irradiated seeds planted at the California Institute of Technology. One Group 2 allele, w_{8624}, has been found.

TABLE 15

Summary of three point data from testcrosses involving cl_1

Genotype of F_1	Parental combinations		Recombinations Region 1		Region 2		Region 1 and 2		Totals	Percent recombinations Region 1	Region 2
$\frac{ra_2\ +\ +}{+\ cl_1\ T3\text{-}9c}$	31	45	5	9	0	0	0	0	90	15.6	0
$\frac{+\ +\ lg_2}{cl_1\ T3\text{-}9c\ +}$	34	36	2	3	4	4	0	0	83	6.0	9.6
$\frac{ra_2\ +\ +}{+\ cl_1\ lg_2}$	61	60	8	9	39	23	2	2	204	10.3	32.4

TABLE 16

Summary of two point data from testcrosses involving cl_1

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{+\ T3\text{-}9c}{cl_1\ +}$	189	190	11	8	398	19	4.8
$\frac{+\ +}{cl_1\ T3\text{-}9c}$	62	61	3	2	128	5	3.9
					526	24	4.6
$\frac{ra_2\ +}{+\ cl_1}$	213	248	32	30	523	62	11.9
$\frac{+\ lg_2}{cl_1\ +}$	79	98	26	26	229	52	22.7
$\frac{+\ +}{cl_1\ lg_2}$	68	70	25	41	204	66	32.4
					433	118	27.3

Indications that this locus was linked with waxy in the presence of *T3-9c* were obtained on F_2 ears, suggesting that this gene was on chromosome 3. Testcross data (Table 17, first cross) with this translocation indicate that w_{7748} is 20.3 units from the break point in chromosome 3.

Heterozygous w_{7748} plants when pollinated by *B-3b* produced kernels that gave rise to albino seedlings. Thus, this gene must be located in the distal nine tenths of the long arm of chromosome 3, the portion indicated by ROMAN and ULSTRUP (1951) that is attached to the B centromere in this translocation. Since LONGLEY (1958) has placed the break point of *T3-9c* at 3L.09 (approximately the same as that of *B-3a*) and since 20.3 percent recombination is observed with this translocation, w_{7748} must be distal to *T3-9c*.

TABLE 17

Summary of two point data from testcrosses involving w_{7748}

Genotype of F_1	Parental combinations		Recombinations		Totals	Total recombinations	Percent recombinations
$\frac{T3\text{-}9c\ +}{+\ w_{7748}}$	67	82	22	16	187	38	20.3
$\frac{+\ lg_2}{w_{7748}\ +}$	73	91	10	12	186	22	11.8
$\frac{ra_2\ +}{+\ w_{7748}}$	63	80	28	41	212	69	32.5
$\frac{ts_4\ +}{+\ w_{7748}}$	65	63	13	19	160	32	20.0

Table 17 (second–fourth crosses) gives the results of two point tests with lg_2, ra_2 and ts_4. Using the data from these linkage studies the following map can be constructed: ra_2–12.5–ts_4–20.0–w_{7748}–11.8–lg_2.

Pastel$_{8549}$: This Group 3 mutant was found in the progeny of a seed grown at the California Institute of Technology that was exposed to a gamma ray dose of 40,000r units. This mutant and its allele, white mutable, which have been described by ROBERTSON and ANDERSON (1961) are allelic to y_1. Since all linkage tests have given results consistent with the known linkage relationships of the y_1 locus on chromosome 6 no linkage data for these pastel alleles will be given here.

Albescent: This Group 3 mutant was first described by PHIPPS (1929) and placed on chromosome 2 by PERRY and SPRAGUE (1936). In his linkage map, RHOADES (1954) has placed it two units proximal to ws_1 and seven units distal to lg_1 on chromosome 2. No further linkage data on this mutant are available at the present time.

SUMMARY

Available linkage data for 13 pigment deficient mutants of maize are summarized. The results of these studies are tabulated below (Linkage information given is based on data from this report unless indicated otherwise):

vp_2	chromosome 5:	a_2–3.3–vp_2–0.9–ps–3.3–bm_1
vp_5	chromosome 1:	sr–0.8–vp_5–23.4–ts_2
vp_9	chromosome 7:	vp_9–10.9–gl_1–17.9–$T7$-$9a$
ps	chromosome 5:	See vp_2
w_3	chromosome 2:	v_5–23.9–w_3–33.8–Ch
lw_1	chromosome 1:	Kn–(1.1)–lw_1–23.8–bm_2
lw_2	chromosome 5:	pr–2.2–lw_2gl_8–18.3–$T5$-$9a$
cl_1	chromosome 3:	ra_2–11.9–cl_1–27.3–lg_2
w_{7748}	chromosome 3:	ts_4–20.0–w_{7748}–11.8–lg_2
pas_{8549}	chromosome 6:	Allele to y_1
al	chromosome 2:	ws_3–4–al–7–lg_1 (RHOADES 1954)
lw_3	chromosome 5:	Duplicate factor with lw_4. To right of bt, in long arm (TULPULE 1954)
lw_4	chromosome 4:	Duplicate factor with lw_3. su_1–13–lw_4–33–gl_3 (TULPULE 1954)

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THE EFFECT OF CARBON MONOXIDE AS A RESPIRATORY INHIBITOR ON THE PRODUCTION OF DOMINANT LETHAL MUTATIONS BY X-RAYS IN DROSOPHILA[1]

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THE modifying influence of carbon monoxide on the extent of X-ray-induced genetic damage in Drosophila has been studied in the past by HAAS, DUDGEON, CLAYTON and STONE (1954); STONE, HAAS, ALEXANDER and CLAYTON (1954); ALEXANDER and STONE (1955); FRITZ-NIGGLI (1959); CHANG, WILSON and STONE (1959); and OSTER (1959). Irradiation, delivered in an environment of carbon monoxide, with or without intentional addition of a few percent of air or oxygen, regularly gave a higher damage than if an inert gas was used instead of carbon monoxide. This refers to the production of dominant and recessive lethal mutations and of translocations. Working with root tip cells of *Vicia faba*, KIHLMAN (1958) has reported a great influence of very small amounts of oxygen on X-ray-induced breakage of chromosomes, if respiration of the cells was blocked by respiratory inhibitors. Carbon monoxide is such a respiratory poison, blocking cytochrome oxidase (a light reversible reaction).

During recent years another role of the respiratory enzymes in connection with radiation effects came into the foreground, a role concerning chromosomal reunion processes, restitution and recombination of broken chromosomes. Most of this work has been done with plant material, but it has been shown that these processes also play a role in Drosophila in oöcytes and in early spermatids, (OSTER 1955; HERSKOWITZ and ABRAHAMSON 1956; ABRAHAMSON 1959; PARKER 1959; FALK 1959). It seems to be a well-established fact that chromosomal reunion processes require a supply of energy which normally is dependent on an undisturbed activity of enzymes engaged in respiration. In this connection BEATTY and BEATTY (1960), using microspores of Tradescantia, published results indicating that carbon monoxide in a mixture with five percent oxygen exerted an influence only on the reunion processes leading to different amount of chromosomal interchanges. In this test, the amount of primary breaks following treatment in carbon monoxide was the same as the amount following treatment in helium plus five percent oxygen. This is in contrast to the findings obtained with Drosophila, where for the production of dominant lethals, carbon monoxide has an enhancing effect if present during irradiation but where no posttreatment effect could be shown (ALEXANDER and STONE 1955). The findings of KIHLMAN (1958)

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and of BEATTY and BEATTY (1960) made it desirable to investigate the situation further in Drosophila.

In order to do this, we performed the following experiments:

Test 1. To prove or disprove the assumption that previous posttreatment experiments had been too short—ALEXANDER and STONE (1955) had posttreated for 30 minutes—we applied a very long posttreatment of nine hours.

Test 2. In order to test the influence of small amounts of oxygen with blocked enzymes, we compared the dominant lethal rate obtained by 1000r in an environment of tank-CO with the rate obtained in CO + 2% O_2 and in He + 3% O_2, at a temperature of 22°C.

Test 3. In this test we wanted to see if it was possible to show an effect of the small oxygen impurities which are contained in commercial carbon monoxide. We compared the dominant lethal rate obtained by 1000r in an environment of carbon monoxide (indicated minimum purity: 99.5 percent with the rate obtained in helium of a minimum purity of 99.99 percent, the irradiation being done at a temperature near the freezing point in order to eliminate enzyme activity in both cases.

MATERIALS AND METHODS

Young males of *Drosophila virilis*, Mexico strain (Texmelucan), were irradiated at an age of 12–24 hours. Afterwards they were aged for five days (adult males of *D. virilis* require six days to mature) and then were mated individually to three virgin females from a cross of "Argentina strain" × "Brazil strain" in order to obtain maximum heterosis. Every two days for a period of from ten to 18 days the males were remated to three new females. From each mating one or two females were separated, and all eggs laid on the second, third and fourth days after the mating period were counted. The percentage of dominant lethal mutations was determined from the proportion of eggs which failed to develop into pupae.

The X-rays were obtained from a constant potential machine (250 kv; 15 ma; filter: 0.5 mm Cu + 1 mm Al). The dose rate was 330r/min and the doses were measured with a Victoreen-r-meter. The flies were irradiated always in the same plexiglass container of 22 mm diameter and a height of 5 mm which could be closed gas-tight. The desired gas was administered to the flies through an inlet and an outlet in the bottom. In each series 50 males were treated simultaneously.

In test 1a (CO-posttreatment) and 1b (control), irradiation with 500r was performed in air at a temperature of 22°C. The flies which were posttreated with carbon monoxide were transferred to a larger container and exposed to a mixture of 95% CO + 5% O_2 within 60 seconds after irradiation. Under this treatment the flies remain completely motionless. They were stored, together with the control group in air, in the dark for nine hours.

In tests 2a, 2b, and 2c, X-ray doses of 1000r were given at 22°C. In 2b, tank-CO was flooded through the container for six minutes, and the flies were held in that gas for an additional six minutes before irradiation. In test 2a, 120 cc of a gas

mixture of tank-CO + 2% O_2 were flooded through the container before it was closed; the flies were held in this mixture for 12 minutes. The pretreatment and the irradiation were given in both cases in the dark. The containers were opened four minutes after irradiation. In test 2c, 120 cc of the gas mixture of He + 3% O_2 were used, but the pretreatment in this case was reduced to two minutes in order to prevent a significant amount of oxygen consumption before irradiation.

In test 3, irradiation with 1000r was done (3b) in helium (indicated purity 99.99 percent minimum) and (3a) in tank-CO (purity 99.5 percent minimum). The respective gas was flooded through the container for two minutes; then the container was submerged in ice water for 12 minutes after which the irradiation dose was immediately administered in a precooled environment and in the dark. The containers were opened four minutes after irradiation.

For the purpose of comparison it is advantageous to plot the obtained values in a curve, although one must be aware that the connecting lines have no meaning but are just a visual help. In our nomenclature "A" means sperm irradiated 5–7 days before fertilization, "B", 7–9 days and so on. It is not possible to attach all these letters to a certain stage of spermatogenesis, because the different treat-

TABLE 1

The frequency of dominant lethal damage in Drosophila virilis *in different stages of spermatogenesis after irradiation under various conditions. See text and Figures 1–3 for other information*

			A	B	C	D	E	F	G	H	I
1a	500r, posttreat. in CO	Eggs	1023	1427	1247	697	1157	853	599	506	689
		Pupae	842	1187	645	282	693	690	511	487	670
		Lethals, %	17.7	16.8	48.3	59.5	40.1	19.1	14.7	5.5	2.7
1b	500r, control	Eggs	1537	3035	1542	1259	1459	971	1504	550	275
		Pupae	1333	2212	710	508	907	791	1353	519	268
		Lethals, %	13.3	27.1	54.0	59.7	37.8	18.5	10.0	5.6	2.5
2a	1000r, in CO + 2% O_2	Eggs	944	997	596	818	166	779	734	...	...
		Pupae	747	653	226	180	46	438	541	...	...
		Lethals, %	20.9	34.5	62.1	78.0	72.3	43.8	26.3	..	..
2b	1000r, in CO	Eggs	877	962	468	638	237	796	659	...	...
		Pupae	694	686	267	306	135	581	580	...	...
		Lethals, %	20.9	28.7	43.0	52.0	43.0	27.0	12.0	..	..
2c	1000r, in He + 3% O_2	Eggs	865	747	948	617	1089	...	...	...	...
		Pupae	690	528	444	350	820	...	...	...	...
		Lethals, %	20.2	29.3	53.2	43.3	24.7	..	..	..	..
3a	1000r, in tank-CO	Eggs	1649	1446	941	547	386	582	...	...	...
		Pupae	1269	1100	518	300	301	482	...	...	...
		Lethals, %	23.0	23.9	45.0	45.2	22.0	17.2	..	..	..
3b	1000r, in He	Eggs	1692	1074	879	908	1120	619	...	...	...
		Pupae	1365	849	496	537	902	533	...	...	...
		Lethals, %	19.3	21.0	43.6	40.9	19.5	13.9	..	..	..

ments sometimes have a different influence on the speed of spermatogenesis. It is therefore necessary to look at the curve as a whole and not to put too much emphasis on the values obtained in a single mating period.

RESULTS

In test 1, (Table 1, Figure 1) nine hours of posttreatment with carbon monoxide did not influence significantly the dominant lethal rate obtained after a dose of 500r in air. The whole curve 1a is slightly shifted to the right side, due to a delay in the spermatogenesis of the CO-posttreated males.

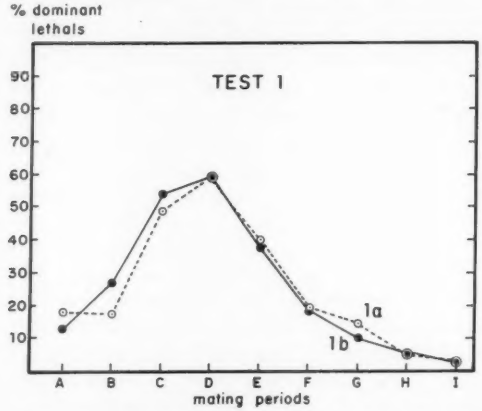

FIGURE 1.—The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis, after an irradiation with 500r X-rays at 22°C. 1a: nine hours of posttreatment with carbon monoxide; 1b: no posttreatment. A: mating period 5–7 days after irradiation, B: 7–9 days, etc.

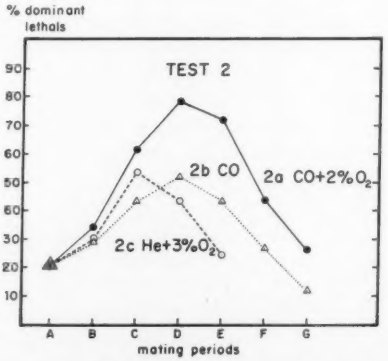

FIGURE 2.—The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis after an irradiation with 1000r X-rays at 22°C in an environment of the indicated gases.

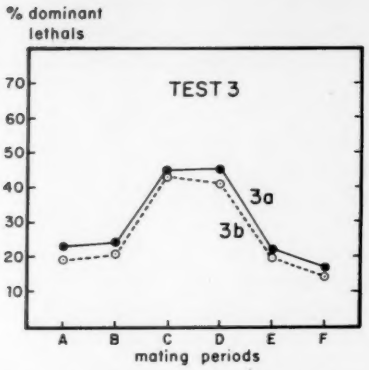

FIGURE 3.—The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis after an irradiation with 1000r X-rays at 0–2°C in an environment of 3a: CO, 99.5 percent pure and 3b: He, 99.99 percent pure.

The result of test 2, (Table 1, Figure 2) is impressive. The difference in the amount of dominant lethal damage caused by the addition of 2% O_2 to the carbon monoxide is very marked. In the sensitive stages of spermatogenesis, in spermatids and spermatocytes, which are represented in the upper part of the curves (Figure 2), over 50 percent more dominant lethal damage is observed in CO + 2% O_2 than in CO alone. The difference dwindles when adult sperm are involved (left end of curve); it is well known, however, that changes in environmental conditions during irradiation have little influence on mature sperm. As a whole the differences are similar to the ones obtained by 1000r and 500r in air with the same dominant lethal system (ALEXANDER, BERGENDAHL and BRITTAIN 1959). Curve 2c (He + 3% O_2) is very similar to 2b (CO), with the exception that the more immature stages are shifted to the left due to the absence of the delaying effect of CO on the speed of spermatogenesis. Curve 2a falls into the range of the results obtained by irradiation with 1000r in air, and the curves 2b and 2c are slightly above the values obtained by the same dose in pure inert gases.

The results of test 3, (Table 1, Figure 3) show (3a) that if the purest degree of commercially available CO (99.5 percent) is used under great care to keep it pure, the effect is a good deal smaller than was usually claimed. On the other hand the comparison with 99.99 percent pure helium (3b) shows that even less than one half percent oxygen present in a gas has a small but clear-cut effect, provided respiration is blocked.

DISCUSSION

The dominant lethal method, in the form in which it is used for these experiments, serves as a good measure of genetic damage in Drosophila. It has been known for several years that spermatids and spermatocytes are much more sensitive to changes of the environmental conditions during irradiation than are

sperm. But, due to the simultaneous influence of the treatments on the speed of spermatogenesis, the results seemed to be very unreliable if one tried to compare the results obtained only at a single point of the curve, that is, at a certain number of days after irradiation. Comparisons using spermatids, therefore, came into an unfavorable light, and sperm, which reacts so poorly, have been the preferred material for a time. A better method is to record the dominant lethal rate obtained in a continuous sequence of mating periods. These entire sequences then can be compared to each other and an eventual shift of the most sensitive stage to an earlier or later mating period is recognizable. This method has been applied with great success especially by M. L. ALEXANDER during the last few years.

Certain difficulties are caused by the occurrence of unfertilized eggs. Most of them can be distinguished easily from dominant lethals if the eggs laid by individual females are counted and recorded separately. If there is no development among large numbers of eggs of a female, or, in exceptional cases, only strikingly few, then this is clearly due to the absence of sperm and such egg counts can be excluded. This is not possible in methods where the egg-laying females are kept together. Unfertilized eggs are a more serious problem than the statistical errors, since the latter become relatively negligible if the values out of which the dominant lethal curves are composed are based on counts of 500–1000 and more eggs.

The kinds of chromosomal breaks that lead to dominant lethals in sperm and spermatids of Drosophila obviously cannot be influenced in the same way as the breaks leading to translocations. Also an extension of the posttreatment with carbon monoxide to nine hours did not bring about additional damage by preventing eventual restitution. The reunion processes following isochromatid breaks in part apparently obey other laws. If it were possible to prevent reunions of the broken chromatids in their original arrangement thereby favoring terminal deletions and the occurrence of sister chromatid unions, one might expect an increased number of dominant lethals. The negative results, so far obtained, have always been based on the one energy-dependent mechanism. It might be possible that another kind of interaction based on a different mechanism of reunion could be successful in changing the fate of broken chromosomes.

The finding of KIHLMAN (1958) that in *Vicia faba*, if respiration is blocked, small amounts of oxygen cause a strong enhancement of the radiation effect applies also to the production of dominant lethal mutations in Drosophila, as we were able to show in tests 2 and 3. For a long time the view was widely held that small amounts of oxygen could be neglected, based on the observations that, if irradiation is performed in an inert gas (N_2, He) and at room temperature, an addition of up to three percent oxygen shows almost no enhancing effect; this is demonstrated once more in our test 2c. Now, it seems to be most probable that under these circumstances the oxygen is removed by respiration before it reaches the chromosomes and their immediate neighborhood within the nucleus. But this enzymatic removal of oxygen stops as soon as the cellular respiration is blocked by respiratory poisons. Figure 4 is an illustration of that mechanism.

Previous theories about protective functions of the cytochrome system become

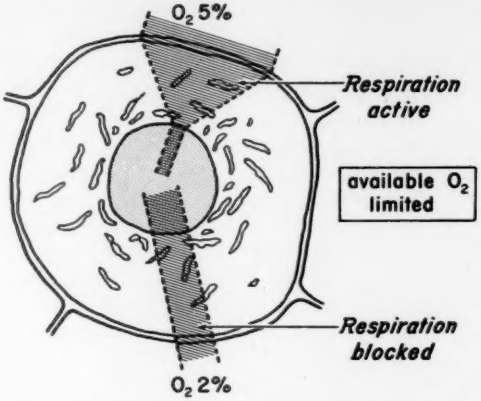

FIGURE 4.—The drawing shows a symbolic cell with mitochondria and the nucleus. If the oxygen supply from outside is limited, respiration at a normal rate removes oxygen before it reaches the nucleus. If the outside oxygen concentration is less than three percent, respiration will remove all oxygen; with five percent oxygen only a very small amount can reach the nucleus. But, when respiration is inhibited, even low concentrations of oxygen gain access to the nucleus and influence chromosome damage by radiation. No quantitative accuracy is intended in this drawing.

superfluous, as is discussed in detail by KIHLMAN, MERZ and SWANSON (1957) and KIHLMAN (1958). It should be mentioned here that STAPLETON, BILLEN and HOLLAENDER (1952) had already described "The role of enzymatic oxygen removal in chemical protection against X-ray inactivation of bacteria." It has taken a long time for all the consequences of the mechanism described in that paper to be recognized and proved for other organisms.

SUMMARY

1. Dominant lethal damage in immature germ cells of *Drosophila virilis*, obtained by X-irradiation in carbon monoxide, is drastically enhanced by the presence of very small amounts of oxygen. This is in accordance with the findings reported for plant material by KIHLMAN where the same effect was shown for chromosomal damage in the presence of several other respiratory inhibitors. Small amounts of oxygen which would easily be removed by cellular respiration get access to the vicinity of the chromosomes if respiration in the cytoplasm is blocked.

2. Posttreatment with CO + 5% O_2 for nine hours in the dark with the flies immobilized by the CO showed no effect on the X-ray-induced dominant lethal rate obtained from mature and immature stages of male germ cells.

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BACK MUTATION IN DROSOPHILA MELANOGASTER. I. X-RAY-INDUCED BACK MUTATIONS AT THE YELLOW, SCUTE AND WHITE LOCI

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THE problem of the X-ray induction of back mutations in Drosophila remains in a rather unsatisfactory state despite some success in inducing back mutations of the forked bristle *(f)* (MULLER and OSTER 1957; GREEN 1959a; LEFEVRE and GREEN 1959) and white-ivory *(w^i)* mutants (LEWIS 1959). Information is still needed to reconcile the findings of TIMOFÉEFF-RESSOVSKY (1931, 1933) and JOHNSON and WINCHESTER (1934) that X-rays can induce back mutation of a number of sex-linked mutants in *Drosophila melanogaster* with the negative findings of several other investigators (see LEFEVRE 1950; LEFEVRE and GREEN 1959 for review). In brief, does the apparent X-ray reverse mutability of the *f* and w^i mutants represent a special situation, or can selected other mutants reverse just as well? An answer to this question was sought on the assumption that the *f* and w^i mutants are not special and the failure to obtain back mutations means only that mutants capable of reversal have not been studied. As a correlary to this assumption, it is surmised that some (perhaps most) mutants are refractory to reversion by X-irradiation, others are not.

Criteria, therefore, must be established which provide a reasonable basis for uncovering reversible mutants since some mutants are apparently refractory to X-irradiation. Previous experience with the f^{2N} and w^i mutants (GREEN 1959a; LEFEVRE and GREEN 1959; LEWIS 1959) suggested that the following criteria might be gainfully employed. First, it would be most profitable to study those mutants which already have given evidence of spontaneous back mutation. These, presumably, are potentially more likely to back mutate with X-rays than other mutants. Second, premeiotic cells appear to be more prone to the induction of back mutations than meiotic or gametic cells. Consequently, irradiation of females, where primarily premeiotic cells are treated, rather than males appears to be the more advantageous procedure. Irradiation of females has the added advantage in that the majority of induced gross chromosomal rearrangements are eliminated before they are included in gametic nuclei (MULLER 1930). Third, certain technical advantages accrue where sex-linked rather than autosomal mutants are studied. Sex-linked mutants can be included in attached-X chromosomes. When attached-X females homozygous for recessive, sex-linked mutants are irradiated, two treated mutants per locus disjoin together and are scored simultaneously in individual female progeny. In short, each female offspring scored represents two irradiated alleles instead of one by conventional techniques. By and large male progeny may be ignored in the scoring except for the occasional detachments of

X chromosomes transmitted to males. Furthermore, in the attached-X method, recessive suppressor mutations as a cause of phenotypic reversion are, with one possible exception, effectively eliminated. If a frequently mutating suppressor gene is loosely linked to the centromere, such a mutation followed by a non-reciprocal crossover would result in homozygosis of the suppressor mutant and a presumed phenotypic reversal of a mutated gene being studied. Adequate progeny tests of presumed back mutations should effectively exclude this contingency. Dominant suppressor mutants cannot be so readily excluded, but the attached-X method does afford a basis for their detection. Where a genuine back mutation occurs in attached-X chromosomes, usually only one of the two alleles mutates. For any attached-X female carrying a presumed back mutation, it is possible to estimate the frequency of homozygosis of the unaltered mutant among her female progeny from the genetic map position of the locus under study. A dominant suppressor will have its locus either nearer or farther from the centromere than that of the mutants it suppresses. This should lead to a frequency of homozygosis lesser or greater than expected if a genuine back mutation is involved.

Admittedly, this situation provides only presumptive evidence for mutation to a dominant suppressor and more detailed genetic analysis is necessary in order to establish this situation.

Where females are being studied, the possibility that recombination events, such as unequal crossing over, will be misconstrued as back mutations must be considered. For some hypomorphic mutants, it is known that when duplicated they manifest a phenotype which approaches wild type. Ideally, the possibility of unequal crossing over or other similar recombination events is handled by including appropriate marker genes in the genotype. Where mutants are used whose duplication phenotype is known, the phenotype of the reversal can adequately serve to exclude recombination events.

Where suppressor mutations, chromosomal aberrations and recombination phenomena are excluded as causing phenotypic reversion of the mutants under study, it can be concluded that back mutations have been induced (see GREEN 1957 for detailed discussion).

MATERIALS AND METHODS

Initially, the following sex-linked, recessive mutants were selected for study: yellow-2 (y^2) body color, scute (sc) bristles and white-apricot (w^a) eye color. Each has been observed to spontaneously back mutate either to wild type or to a more normal mutant allele. In Table 1, the recorded instances of such back mutations of these three mutants is given. Criteria for establishing the verity of these back mutants are those previously discussed (GREEN 1957) and require no further elaboration here. It will suffice to note that each arose as a single individual where the possibility of contamination has been effectively excluded. In the case of the back mutations of w^a to intermediate states, two types phenotypically distinct from w^a and from each other have also been found. In one the

TABLE 1

Reported spontaneous back mutations of y^2, sc *and* w^a

Mutant	Frequency	Authority
$y^2 \rightarrow y^+$	1	BEDICHEK (1937, Drosophila Inform. Serv. **8**)
$y^2 \rightarrow y^+$	2	GREEN (unpublished)
$sc \rightarrow sc^+$	1	MORGAN, *et al.* (1925)
$w^a \rightarrow w^+$	1	LEFEVRE and FARNSWORTH (1954, Drosophila Inform. Serv. **28**)
$w^a \rightarrow w^+$	1	YANDERS (personal communication)
$w^a \rightarrow w^*$	1	MULLER (1944, Drosophila Inform. Serv. **18**)
$w^a \rightarrow w^*$	1	MOSSIGE (personal communication)
$w^a \rightarrow w^*$	1	MACKENDRICK (1958, Drosophila Inform. Serv. **32**)
$w^a \rightarrow w^*$	3	GREEN (unpublished)

w^* = intermediate allele.

eye coloration is maroon-like while in the other the coloration is reddish approaching wild type in appearance. A detailed study of these phenotypes is reported elsewhere (RASMUSON, GREEN and EWERTSEN 1960). In addition the following mutants were studied: yellow-1 (y^1) allelic to y^2; white-apricot-2 (w^{a2}) and white-apricot-4 (w^{a4}) allelic to w^a; w^{aRM}, a spontaneous partial back mutation of w^a found by MOSSIGE, and a white eye color mutant w, pseudoallelic to w^a. Insofar as is known, spontaneous back mutations of these mutants have not been demonstrated. However, JOHNSON and WINCHESTER (1934) reported one X-ray-induced back mutation of y (presumably y^1), and some reversions of w^{a2} to w^+ interpreted as being the consequence of double crossing over in a short interval could conceivably be back mutations (GREEN 1960a).

A number of homozygous attached-X stocks were synthesized using a triploid stock, constituted of attached-X chromosomes homozygous for y^2 *sc* w^a *ec* and a free X balancer chromosome. Attached-X stocks of the following genotypes were obtained: y^2 *sc* w^a *ec* (*ec* = echinous eyes); y^2 w^{a2} (y^2 derived from a stock supplied by DR. J. SCHULTZ); y^1 w^{a4}; w^a ct^n sn^3 (ct^n = cut-notch wings; sn^3 = singed-3 bristles); y^2 *sc* w^{aRM} (y^2 *sc* from y^2 *sc* w^a *ec* stock). In each attached-X line, males of the genotype y^2 *sc* w *ec* or y^2 *sc* w^a *ec* were used, these serving for the determination of the spontaneous back mutation roles of each of the mutants. Back mutations of *ec* were not sought.

Females of the appropriate genotype were irradiated with 5,000r X-rays (90 kv, 3 ma) delivered at a rate of approximately 300r/minute. X-ray output was checked with a Victoreen dosimeter and was found to vary by ± ten percent. Since attached-X chromosomes are matroclinous in inheritance, the need for collecting virgin females was obviated. Females 0–48 hours were irradiated and 20 treated females with an excess of males were mated in half-pint milk bottles containing the standard corn meal agar medium. Females were allowed to oviposit for six days, transferred to fresh media for a second interval of four days followed by a third interval of four days after which they were discarded. All flies were raised at a controlled temperature of 23–24°C.

In scoring, all female progeny were counted while males were only examined.

The number of males and females was assumed to be equal. Where y^2 and *sc* were scored, only reversals to wild type were sought. Undoubtedly partial reversals occur but their discrimination is at best difficult and time consuming. As will be noted later, one partial reversion of *sc* was found. Where the eye color mutants were examined partial and complete reversals were sought. Very likely, because of the problem of visual acuity and the separation of small phenotypic effects, not all possible partial back mutations of eye color mutants were found. Therefore, these back mutation rates must be recognized as minimal.

Where females, and therefore primarily premeiotic cells, are being irradiated, consideration must be given to the possible effect "clusters" will have on the estimation of the frequency of back mutation. This question requires some discussion for the added reason that the progeny of 20 irradiated females were scored per bottle. Previous experience indicates that back mutations are at best quite rare such that among the progeny of 20 females it is highly unlikely that more than a single back mutation will occur. Where more than one back mutation per bottle is recovered, it can be safely assumed that back mutation occurred as a premeiotic event sufficiently early as to recur in several subsequent female gametes. Therefore, it was arbitrarily decided that no more than one back mutation per bottle was likely to occur. By this decision, the mutation rates will be under rather than overestimated.

Each female with a reversion phenotype was progeny tested to prove the germinal nature of the reversion. Additional crosses designed to assess the genetic nature of the back mutations were made and will be described, where appropriate, below.

RESULTS

The results of five separate sets of experiments are tabulated in Table 2 and establish the following points. First, independent reversions of y^2 and *sc* to alleles producing a wild-type phenotype were induced by X-rays. Successful progeny

TABLE 2

Recovery of back mutation after irradiation of attached-X females (dose = 5000r)

Genotype attached-X female	Number of back mutations y^+	*sc*	w^+	Total chromosomes
y^2 *sc* w^a *ec*	2	3	2	100,394
y^2 w^{a2}	10	.	.	104,884
y^2 *sc* w^{aRM}	7	9	1	99,284
w^a ct^n sn^3	.	.	2	90,568
y^1 w^{a4}	.	.	.	110,370
y^1 w	.	.	.	104,608

Back mutation rate:
$y^2 = 19/304{,}562 = 6.24/10^5$ chromosomes
sc $= 12/199{,}678 = 6.01/10^5$ chromosomes
$w^a = 4/190{,}962 = 2.09/10^5$ chromosomes

tests were obtained on each of the 19 y^2 and 12 *sc* reversals. These tests showed that all of the reversals are germinal and involved one of the two irradiated alleles. Where y^2 reverted, *sc* was unaffected and *vice versa*. The frequency of homozygosis of the mutant allele among the female progeny was determined for each reversion. Since y^2 and *sc* are located approximately 60 units from the centromere, a homozygosis frequency approximately 25 percent is expected assuming no interference and no chromosomal aberrations. This expectation was fulfilled in each case as exemplified by typical progeny tests listed in Table 3. These results also support the contention that dominant suppressor mutations are very likely not involved provided it is assumed that such suppressors are not tightly linked to either reverting locus. The fact that the y^2 and *sc* reversals occurred independently of one another plus their extremely tight linkage makes it most unlikely that unequal crossing over and subsequent duplication of y^2 or *sc* is involved in the phenotypic reversions. If unequal crossing over and duplication are involved, the extremely tight linkage of y^2 and *sc* would mean that both would be duplicated and therefore revert simultaneously. Since no such case was found, this explanation is unlikely and unnecessary.

While the bulk of the y^2 and *sc* back mutants occurred as single females, three separate instances of y^+ clusters were found among the progeny of irradiated $y^2\ w^{a2}$ females. In one case, six y^+ females were found in a single bottle; and in two cases, two y^+ females per bottle. These were all interpreted as single reversal events. Progeny tests of the cluster of six females are of interest because they show that four of the females were heterozygous for the reversal, two were homozygous. This is expected where the reversion is premeiotic.

In addition to the phenotypically complete *sc* reversals, one partial *sc* reversal was found. This was first detected as phenotypically wild type, but the subsequent progeny test showed that this reversal did not invariably produce a wild-type phenotype either when homozygous or heterozygous with *sc*. In all likelihood other partial reversals were overlooked since no systematic search for them was made.

There can be little doubt that X-irradiation has significantly increased the

TABLE 3

Representative progeny tests of females phenotypically y^+ *or* sc^+

Phenotype reversal female	Phenotype female progeny	
	y^+	y^2
1. $y^+\ sc\ w^a\ ec$	39	7
2. $y^+\ w^{a2}$	88	22
3. $y^+\ w^{a2*}$	68	14
4. $y^+\ sc\ w^{aRM}$	90	21
	sc^+	*sc*
1. $y^2\ sc^+\ w^a\ ec$	35	12
2. $y^2\ sc^+\ w^a\ ec$	32	10
3. $y^2\ sc^+\ w^{aRM}$	64	15

* Spontaneous back mutation y^2.

back mutation rate of both y^2 and *sc*. In view of the exceedingly rare occurrence of spontaneous back mutations, the mere fact that a measurable back mutation frequency of both y^2 and *sc* has been recorded is sufficient to warrant this conclusion. Of interest is the fact that during this study one instance of spontaneous back mutation was found in the stock from which attached-X y^2 w^{a2} females were obtained for irradiation. The progeny test of this female, included in Table 3, showed that one of her two y^2 mutants reverted. Note also that the homozygosis frequency among her progeny is identical to that of the induced reversals. Using this stock of females attached-X y^2 w^{a2} crossed to w males, a total of 269,114 y^2 w^{a2} chromosomes were scored and no spontaneous reversals were found. Among the unirradiated control males in the irradiation experiments, approximately 200,000 individuals carrying y^2 and *sc* were scored, and no spontaneous reversals were found.

From irradiation experiments designed for quite another purpose (GREEN 1960b) it was possible to extract some information bearing on the question of the radiosensitivity of gametic *versus* pregametic cells. In these experiments the main objective was the determination of the X-ray mutation rates of white (w^+) loci of two dissimilar stocks. Males y^2 z (z = zeste eye color) carrying one or the other w^+ allele were irradiated and crossed to females homozygous y^2, w^a, v (vermilion eye color) and f included within a double inversion complex. They were allowed to mate with the same females over three separate four-day egg-laying intervals. Since there was a marked drop in the number of progeny produced in the third four-day interval, it seems reasonable to conclude that most of the progeny were derived from irradiated sperm. Among a total of 154,913 F_1 females scored, only one rather unusual reversal that of y^2 to y^+ was found. This reversal occurred as a single y^+ female of presumed genotype y^2 w^a v f/y^+z. On testing this female produced three kinds of male offspring: y^2 w^a v f; y^+ z; y^1 z. A possible interpretation for this situation is that on irradiation, a break occurred at the y^2 locus which did not immediately repair. The nature of the repair differed in different cells; some repaired to y^+, others to a mutated form identical to the allele y^1. Both types occurred among the ovaries of the F_1 female. Clearly back mutations of y^2 to y^+ in irradiated sperm occur less frequently than in premeiotic female cells.

The results of JOHNSON and WINCHESTER (1934), TIMOFÉEFF-RESSOVSKY (1932) and GOLDAT (1936) are particularly relevant here. These investigators studied back mutation in males treated with approximately 4000r. All recorded back mutations of *sc* to sc^+ in frequencies as follows: TIMOFÉEFF-RESSOVSKY, 3/17,676; JOHNSON and WINCHESTER, 1 + 2?/101,042; GOLDAT 1/37,766. (Those marked "?" are unproved.) These data, considered together, suggest that induced reversals of *sc* in sperm are indeed rare, appreciably rarer than reversals in premeiotic female cells. In this respect the results for y^2 and *sc* agree with similar observations made on back mutation of the f^{3N} (LEFEVRE and GREEN 1959; GREEN 1959a) and w^1 (LEWIS 1959).

In contrast to the comparatively frequent back mutations of y^2, the y^1 allele

has failed to back mutate among 200,000 chromosomes tested under equivalent experimental conditions. Comparable results were reported by GOLDAT (1936) who found no reversals of y (presumably y^1 since it is described as a yellow bristle, and body color mutant) among 133,657 irradiated male gametes scored. TIMOFÉEFF-RESSOVSKY (1939) also obtained negative results after irradiation of y males (0/21,897). However, JOHNSON and WINCHESTER (1934) found one y^+ among 69,923 irradiated y gametes tested. Whether this reversal was induced or arose spontaneously is an open question. These essentially negative findings with y^1 are not entirely unexpected especially since apparently no case of spontaneous back mutation of y^1 have been reported. Considering the data as a whole, it seems most reasonable to conclude that in comparison with y^2 the y^1 mutant is refractory to X-irradiation.

Contrary to the back mutation results with y^2 and *sc*, no complete reversals of w^a were obtained despite the fact that two apparent spontaneous back mutations to w^+ have been found (see Table 1). However, as listed in Table 2, four partial back mutations of w^a were recovered. Among 90,943 male controls, no w^a back mutations were found, and it is therefore most likely that X-irradiation has significantly increased the back mutation rate. In phenotype the induced back mutations do not strikingly differ from the spontaneous ones. Among the four induced reversals, one when homozygous resulted in a maroon eye color, while three produced a near wild-type coloration. Each reversal proved on progeny testing to involve one of the two irradiated w^a mutants. From these facts it follows that back mutation rather than unequal crossing over or a recessive suppressor mutation accounts for the altered phenotypes. Unequal crossing over resulting in two w^a mutants linked in tandem on the X chromosome has been detected (GREEN 1959). The eye color phenotype associated with the duplication of w^a, although shifted toward wild type, is in all cases distinguishably lighter than the incomplete back mutations of w^a. Mutation and homozygosis of alleles of the suppressor of w^a (map position in 0.01) might account for the partial back mutations of w^a were it not for the fact that phenotype of suppressed w^a flies is also clearly lighter than that of the reversals. Conceivably a very closely linked dominant suppressor could occur, but crossing over tests with the two w^a reversals occurring in the y^2 *sc* w^a *ec* chromosome failed to uncover such a suppressor. Of interest is the fact that one case of a dominant w^a suppressor was found. This suppressor has not been studied in detail, but it is clear that it is localized some distance to the right of w^a and is lethal when homozygous. Further studies of this dominant suppressor are underway.

The recovery of only partial reversals of w^a suggested that possibly a situation comparable to the back mutations of w and w^e (white-eosin) as reported by TIMOFÉEFF-RESSOVSKY (1933) was involved. He reported that from irradiation of w males only partial back mutations were recovered, whereas following irradiation of w^e males, reversions to wild type did occur. Therefore, irradiation experiments were carried out with attached-X females homozygous for the spontaneous partial back mutation w^{aRM} on the assumption that it would more

likely revert to wild type than w^a. These expectations were not borne out. As listed in Table 2, the only back mutation found was a further reversion toward but distinguishable from wild type. This reversal was phenotypically inseparable from several of the partial back mutations of w^a. The finding of one partial reversal of w^{aRM} should not be construed as a quantitative recovery of these mutants since great difficulty was encountered in objectively distinguishing females homozygous w^{aRM} from females heterozygous for w^{aRM} and the phenotypically nearer normal reversals.

Although no reversals of w^{a2} were found, one genetical event of some interest was noted. On four separate occasions y^2 females of a rather deep brownish eye color were recovered among the progeny of attached-X y^2 w^{a2} females. Progeny tests gave identical results: all the female progeny were y^2 with the parental deep eye color; no w^{a2} females were observed among large numbers of progeny. At first glance this seemed like four separate instances of partial back mutation of w^{a2}. On further consideration this interpretation was considered improbable since it necessitated the unlikely coincidence that all four presumed reversals must be accompanied by crossing over and homozygosis subsequent to mutation. A more reasonable and testable interpretation was developed from the observation that the coupled genotype w^{a2} *rb* (ruby eye color) is *rb* in phenotype. Since *rb* is localized at 5.5 on the X and is among the most frequently mutating of the X chromosome loci (VALENCIA and MULLER 1948), it was deemed more likely that the presumed w^{a2} reversals were in fact cases of induced premeiotic mutations to *rb* alleles followed by crossing over and homozygosis. This explanation was tested by obtaining detachments of the attached-X chromosomes and crossing to a known *rb* allele. Fulfilling expectation, all four presumed w^{a2} reversals proved to carry homozygous *rb* mutants.

The data for the mutants w^{a4} and w are self-explanatory and need no further elaboration. Within the scope of the experiments, it is concluded that these mutants are refractory to X-irradiation. One occurrence of a recessive, partial suppressor of w^{a4} was found. This suppressor mutation, although incompletely studied, is sex-linked and localized an appreciable genetic distance to the right of w^{a4}.

In previous experiments where a comparatively high induced back mutation rate of f^{1N} was observed (approx. 1/2000 after 4200r), it was suggested that such reversion rates are not inconsistent with a recombination type event (LEFEVRE and GREEN 1959). The precise mechanism whereby recombination would simulate back mutation is, however, not clear. Nonetheless an experiment was undertaken to determine whether a mutant will back mutate in a genotypic situation where recombination between homologous chromosomes has been effectively eliminated. Such recombination, conventional or unconventional, is not expected between a mutation and its deficiency. Back mutations were, therefore, sought among the progeny of irradiated females possessing a normal X chromosome of the genotype y^2 *sc* w^{aRM} and an X chromosome carrying a deficiency for y^+ as well as the adjacent loci. The deficiency selected was *Df(1)sc*^{8}. This X chromo-

some is the sc^8 inversion in which a deficiency for y^+ and ac^+ loci was induced (STURTEVANT in BRIDGES and BREHME 1944). The deficiency superimposed on the inversion effectively excludes genetic exchange between the y^2 loci since one chromosome carries no y^2 locus. Although sc^8 carries a sc^+ locus the presence of a deficiency plus inversion almost entirely eliminates recombination. Thus any back mutations of y^2 cannot be and of *sc* almost certainly are not ascribable to recombination events.

Females y^2 *sc* $w^{aRM}/Df(1)sc^8$, w^a were irradiated with 5000r and crossed to males y^2 *sc* w^e, as described above. (The w^{aRM} mutant served to differentiate between the intact and deficiency X chromosomes.) Back mutations of y^2 were sought among all F_1 progeny carrying the y^2 *sc* w^{aRM} chromosome; those of *sc* only among males. Back mutations as follows were recovered: 2 y^+/40,840 treated chromosomes; 1 sc^+/19,778 chromosomes. All back mutations were proved to be germinal by progeny testing. One additional sc^+ male, not included in the summary, was sterile. No reversals of w^{aRM} were found. These back mutations mean that it is highly unlikely that back mutation occurs as a consequence of some type of recombination between homologous chromosomes.

While the frequency of homozygosis in attached-X females suffices in determining whether back mutants are or are not associated with comparatively gross chromosomal rearrangements, it cannot be gainfully employed for detecting "minute" rearrangements. For the latter either detailed cytological analysis or crossing over in the immediately adjacent regions is necessary. Crossing over between pseudoalleles appears to be the most sensitive indicator of "minute" structural changes and therefore two of the four back mutations of w^a were so tested with the mutant w^{ch}, pseudoallelic to w^a. In the presence of heterozygous autosomal inversions, crossing over between the back mutants and w^{ch} occurred at a frequency equivalent to that between w^a and w^{ch}. This demonstration of the disassociation of the back mutants with chromosomal rearrangements is not unexpected since it has been the general experience that most mutants, spontaneous or induced, either permit recombination to occur freely or markedly influence the frequency of crossing over in their immediate vicinity.

For testing the y^2 and *sc* back mutations, a slightly different and rather less sensitive crossing over procedure was adopted. This involved determining crossing over between *y* or *sc* and *su-*w^a, a tight linkage. The crossover frequency between y^2 and *su-*w^a is around 0.01 percent and can be increased to approximately 0.5 percent through the enhancing influence of the heterozygous autosomal inversions *Cy* and Ubx^{130}. Each of four sc^+ and three y^+ back mutants was made heterozygous for a marked X chromosome of the genotype y^1 *sc su-*w^a w^a *spl* and the autosomal inversions *Cy* and Ubx^{130} and crossovers between y^1 and *su-*w^a were sought. It will suffice here to note that for each back mutation tested the desired crossovers were readily recovered. That crossing over in the *y–su-*w^a interval is a somewhat insensitive indicator of "minute" rearrangements follows from tests where the "minute" rearrangements sc^{10} and sc^{260-22} were compounded to the aforementioned marked X chromosome. Cytologically, sc^{10} is associated

with a tiny inversion extending from the right of band 1B2 (the *sc* locus) to just to the left of band 1C1 (MULLER, PROKOFYEVA and RAFFEL 1935), while sc^{260-22} is a slightly longer inversion with a comparable right break but a left break between 1E1,2 and 1E3,4 (SUTTON 1943). Where crossing over with sc^{10} was studied approximately 0.2 percent crossing over between *y* and *su-w*^{a} was detected among slightly more than 2,000 individuals. With sc^{260-22} no crossovers in the *y–su-w*^{a} interval were recovered among 2,366 individuals scored. These observations mean that the y^+ and sc^+ back mutations are at the utmost "minute" rearrangements of the sc^{10} type.

DISCUSSION

A number of conclusions are warranted by the experimental data presented. First, the recovery of X-ray-induced back mutations of the y^2, *sc* and *w*^{a} mutants at measurable rates means that X-rays can act as a potent mutagen for this process in Drosophila. Furthermore, these findings mean that the mutants f^{3N} and w^i, previously shown to be reversible by X-rays, are neither special nor unusual. There are clearly two classes of mutants in Drosophila: those that readily back mutate and those that do not following X-ray treatment. Furthermore, the experimental data submitted here show that the back mutation process must be delimited to the mutant gene itself and is not confounded by recombination events either of the usual or unusual types.

Although three of the mutants studied here—y^1, w^{a2} and w^{a4}—appear to be refractory to X-rays, this conclusion must be tempered with reservation. It is self-evident that the detection of back mutations is, in part, a function of the number of treated cells which can be objectively and efficiently scored. Drosophila is clearly not the organism of choice where information is sought on the variability or limits of back mutation rates of independent and diverse mutants. In this respect the use of nutritionally deficient mutants in microorganisms where selection techniques may be exploited is superior. Using such techniques GILES and his colleagues (GILES 1951, 1956; GILES, DE SERRES and PARTRIDGE 1955; DE SERRES 1958) have detected X-ray-induced back mutation rates of Neurospora mutants as low as 0.1×10^{-7} surviving treated conidia using X-ray doses as high as 34,000r. It goes without saying that the detection of mutation rates at this or even somewhat greater orders of magnitude is out of the question for Drosophila. Hence, the failure to detect back mutation in Drosophila need not mean that the mutant under study is refractory to X-rays, but rather that the induced rate is so low as to defy detection by conventional techniques.

One question which merits some discussion is the following. Are all the y^2 and *sc* back mutations identical or do they represent a family of genetic changes all capable of producing a wild-type phenotype? The detection of so-called wild-type isoalleles as the *ci* and *w* of loci in Drosophila (STERN and SCHAEFFER 1943; MULLER 1935; GREEN 1959b) favors the idea that back mutants represent a group of nonidentical isoalleles. This is supported by the detection of a number of wild-type isoalleles at the *sc* locus (STURTEVANT, personal communication).

Where back mutations can be studied at the biochemical (enzymatic) level, as in Neurospora, convincing data have been adduced to show that the back mutants form a family of nonidentical genetic changes (GILES 1958; WOODWARD, PARTRIDGE and GILES 1960; ESSER, DE MOSS and BONNER 1960). In this connection an attempt was made to discriminate among the y^a back mutations by taking advantage of an observation made by SACHAROV (1936) that the sex-linked mutant divers (*dvr*) manifests a curled wing phenotype only when simultaneously homozygous for a y mutant. Among ten y^+ reversions tested all behaved identically in failing to elicit the *dvr* phenotype. While this demonstrates that insofar as *dvr* is concerned the back mutants are inseparable, it does not exclude the possibility that when more objective criteria are employed, separation will be made.

SUMMARY

1. The induction of back mutations by X-rays was studied using seven independent sex-linked mutants in *Drosophila melanogaster* and irradiating attached-X females.

2. Significant increases in the back mutation rates of the mutants y^a, *sc* and w^a were found.

3. No evidence was found that the back mutations were caused by recombination events or mutations to independent suppressors.

4. Crossing over tests indicate that the back mutations are not associated with chromosome rearrangements.

5. The problem of X-ray induction of back mutations is briefly discussed.

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SOME BIOCHEMICAL FACTORS IN X-RAY-INDUCED MUTATION IN BACTERIA[1]

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SEVERAL years ago WITKIN (1956, 1958) presented evidence which suggested that, in the case of ultraviolet light (UV) induced mutation in bacteria, "the time interval between the absorption of radiant energy and the production of stable genetic change can no longer be regarded as infinitesimal." She pointed out that many postirradiation treatments alter the ultimate fate of a potential genetic change. WITKIN suggested that protein synthesis is involved in mutation induction by UV since she found that amino acid supplementation increases mutation frequency response and that chloramphenicol, an antibiotic which inhibits protein synthesis, appreciably lowers the mutation frequency obtained with a given dose of UV. This type of postirradiation response was also found with ionizing radiations (KADA, BRUN and MARCOVICH 1960) as would be predicted by the existence of an appreciable "phenotypic" delay in X-ray-induced mutation (DEMEREC and LATARJET 1946). It is evident that the involvement of protein synthesis in mutagenesis must be restricted to certain mutation induction mechanisms since other mutations are not affected by identical postirradiation treatments (WITKIN and THEIL 1960).

HAAS and DOUDNEY (1957) presented evidence suggesting that the chemical basis for UV-induced mutation may involve nucleic acid precursors altered *in vivo* by UV. Subsequent studies by these workers indicate that ribonucleic acid (RNA) synthesis as well as protein synthesis is involved in the mutation induction process. It was found that various postirradiation treatments specifically affecting RNA or protein synthesis are effective in lowering markedly the mutation frequency response to ultraviolet light (DOUDNEY and HAAS 1958, 1959). Treatments of short duration reduced mutation frequency to a low level without significant modification of the time or rate of subsequent syntheses of RNA, deoxyribonucleic acid (DNA) and protein (DOUDNEY and HAAS 1960a,b). The evidence thus suggests that the mutation frequency decline process promoted by these specific agents is an active process, probably enzymatically mediated, and not dependent on delay in gross postirradiation macromolecular synthesis. The progressive loss of susceptibility of the potential mutations to these treatments is closely correlated with the progression of RNA synthesis in the culture. A rela-

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[2] Postdoctoral Research Fellow supported by research training grant CRT-5047 from the National Institutes of Health.

tion exists between the *amount* of RNA synthesized at the time of chloramphenicol addition, the relative *rate* of deoxyribonucleic acid (DNA) synthesis in the presence of chloramphenicol and the induced mutation frequency observed after chloramphenicol challenge. To account for these facts the following hypothesis was proposed (DOUDNEY and HAAS 1960b): Recovery of the capacity to synthesize DNA after UV exposure requires the synthesis of RNA and protein; apparently the necessary RNA and protein must be formed prior to chloramphenicol addition in order for DNA to be replicated with consequent establishment of the mutation in the genome. When this mandatory RNA and protein synthesis has not occurred prior to chloramphenicol addition, the potentiality for mutation is lost, presumably through the active decline process. Thus the synthesis of RNA and protein has an active role in "fixing" the "potential mutation" prior to the synthesis of the new complement of DNA. The nature of the function of the RNA and protein in genetic replication and mutational change remains to be determined.

Recent studies strongly suggest that DNA synthesis is the terminal step in the mutation induction process. Loss of mutation photoreversibility is correlated with DNA synthesis in the culture (HAAS and DOUDNEY 1960; DOUDNEY and HAAS 1960b). It thus appears that the initial synthesis of DNA after UV exposure is the "copying" event leading to a photostable error in the genome. The phenotypic expression of UV-induced mutation to prototrophy is correlated with DNA synthesis in the culture (HAAS and DOUDNEY 1959). Mutation expression will not occur in the absence of DNA synthesis (DOUDNEY and HAAS 1960b). Furthermore, protein synthesis must follow DNA synthesis for phenotypic expression of the induced mutation (HAAS and DOUDNEY 1959). Chloramphenicol blocks mutation expression when added to the culture immediately following the initiation of postirradiation synthesis of DNA while allowing DNA synthesis to proceed. It thus appears probable that the *functional* mutated gene is formed with the initial replication of DNA following UV exposure but that protein synthesis is required for expression of the modified genetic character.

This paper describes research on the induction of mutation by X-rays utilizing techniques similar to those used in the UV-induced mutation studies. The effort is directed toward characterization of the processes involved in X-ray-induced mutation in relation to the postirradiation macromolecular synthetic activities of the irradiated bacteria. Some preliminary aspects of this study have been previously described (KADA, DOUDNEY and HAAS 1960).

MATERIALS AND METHODS

Bacteriological techniques: An auxotrophic strain of *Escherichia coli* (strain WP2) requiring tryptophan for growth was utilized in these studies. This strain (hereafter called the try strain) was kindly provided by DR. EVELYN WITKIN several years ago. In addition, several recently isolated auxotrophic strains requiring various amino acids were utilized. These strains were isolated after UV

irradiation of *E. coli* strain 15 thy, a thymine requiring strain.[1] These strains require (in addition to thymine) either proline, methionine, or both methionine and tyrosine. All strains are kept in stock culture on nutrient agar slants in the cold. Growth of the cultures for experimental purposes is in synthetic media containing 20 μg per ml of the required amino acid and, in case of the thymine-requiring strains, 20 μg per ml of thymine. Growth is for 16 hours at 37°C under vigorous aeration.

The composition of the synthetic minimal medium used is as follows: $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; sodium citrate, 0.5 g; K_2HPO_4, 7.0 g; 1000 ml of water; glucose, 0.2 percent, sterilized separately.

The 16 hour culture is held at 6°C for one hour, for purposes of culture synchronization. The bacterial cells are then washed two times, by centrifugation and resuspension, with cold 0.9 percent saline and then resuspended in cold minimal medium without glucose (the volume being adjusted to about 1.5×10^{10} cells per ml).

Exposure of the bacteria to X-rays is accomplished as follows: 10 ml quantities of the cell suspension are put into 50 ml Erlenmeyer flasks. Each flask is placed in the middle of a specially designed plastic container and packed in ice. The plastic container is fitted directly over the apperature of the X-ray machine. The radiation factors for the General Electric "Maxitron 250" machine used are as follows: 250 kvp, 30 ma, 1 mm of aluminum added filtration. The dose rate in air at the locus of the cells is approximately 2860r per minute.

The typical experiment is accomplished as follows: The bacterial cells are irradiated, usually with 10 kr of X-rays, resulting in a level of survival of approximately 20 percent. The ice-cold suspension of irradiated cells is then diluted 50 times with fresh, warm (37°C) minimal medium supplemented with a mixture of amino acids (DL-alanine, L-arginine, DL-aspartic acid, L-cysteine hydrochlorate, L-glutamic acid, glycine, L-hystidine hydrochlorate, DL-isoleucine, DL-leucine, L-lysine hydrochloride, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tyrosine, L-tryptophan and DL-valine). Amino acids were present in the liquid medium at a concentration of 10 μg per ml except for the essential amino acids of the strain used (50 μg per ml). The antimetabolites, chloramphenicol (10 μg per ml) or 6-azauracil (50 μg per ml), are added to this medium in several experiments, as indicated. The cultures are then incubated, with satisfactory aeration, in Erlenmeyer flasks placed on a rotary shaker at 37°C. At intervals (usually ten minutes) a portion of the cell suspension is removed, chilled quickly in an ice bath and then washed two times with cold 0.9 percent saline solution. The cells are finally concentrated by resuspending in cold minimal medium (the volume being reduced by twenty to fiftyfold as compared to the irradiated incubation suspension). Samples of the cell suspensions (usually

[1] This strain is commonly referred to in the literature as 15_T^-. The 15 thy designation is adopted in this paper, as being more appropriate to a commonly accepted standard nomenclature in bacterial genetics.

0.1 ml) are plated on two basic sorts of agar medium, for purposes of assaying mutations (HAAS and DOUDNEY 1959):

(a) Minimal agar medium without supplementation. This medium permits the formation of colonies from bacterial cells whose prototrophic character is already established at the time of plating.

(b) Minimal agar medium supplemented with 2.5 percent nutrient broth. This medium permits the metabolic processes involved in mutation induction and depending on amino acids to take place and also provides for the phenotypic expression of the mutated cells through provision of the required amino acid.

In case of the thymineless, amino acidless polyauxotrophs, these media are supplemented with the required growth factors, other than the requirement which is under study for reversion.

All plates are incubated for three days at 37°C and the colonies scored. The frequency of mutation is then calculated, taking into account the titre of viable cells determined on the supplemented agar medium after suitable dilution of the cell suspension, and the spontaneous mutations which exist both in the suspension before irradiation and after residual growth on the mutation assay medium used (either a. or b.). In general, the number of surviving bacteria plated for purposes of scoring induced mutations ranged between 4×10^8 and 7×10^8 per plate for the try strain and between 1×10^8 and 3×10^8 for strain 15 thy met tyr. Identical unirradiated controls were plated in order to determine the number of spontaneously-arising revertant bacteria per plate. This number ranged between 0–10 colonies per plate on minimal agar medium and 10–25 colonies per plate for the minimal agar medium supplemented with nutrient broth. This figure was subtracted from the total number of colonies observed with the irradiated cells (which ranged between 100 and 300 colonies per plate) before calculation of the induced-mutation frequency. Survival is determined by plating at an appropriately higher dilution on minimal agar medium containing 2.5 percent nutrient broth.

Chemical determinations: Culture samples, taken from the incubated suspension for analysis of RNA, DNA and protein are precipitated with 0.25 N perchloric acid in the cold. The nucleic acids are hydrolyzed by incubation in 0.5 N perchloric acid for 50 minutes at 70°C. Analysis for DNA is done by the diphenylamine method of BURTON (1956). For the evaluation of RNA, the ultraviolet absorption at 260 mμ and 290 mμ is determined (VISSER and CHARGAFF 1948), and the amount of DNA, as determined by BURTON analysis of the same sample, is then substracted with correction for extinction coefficients. Protein is determined by the FOLIN method (LOWRY, ROSEBROUGH, FAAR and RANDALL 1951).

EXPERIMENTAL RESULTS

Mutation expression and deoxyribonucleic acid synthesis: HAAS and DOUDNEY (1959) have shown a close correlation following UV exposure of the try strain

between recovery of DNA synthesis and mutation expression as measured by plating on unsupplemented minimal agar medium. They irradiated the cells with UV and then incubated in minimal medium supplemented with casein hydrolysate and tryptophan, the required amino acid. Under these conditions, the delay in DNA synthesis induced by irradiation is of sufficient duration to distinguish the initiation of synthesis of this macromolecule from the initiation of other macromolecular synthetic activities, such as RNA synthesis. By plating at periodic intervals on minimal agar medium, it was determined that a great majority of the ultraviolet-induced mutations become expressed (i.e., capable of forming colonies on minimal agar medium) only following DNA replication.

Experimental conditions which permit the realization of similar experiments in X-ray-induced mutation were devised. Synchronized cultures were irradiated with a dose of ten kr of X-rays and incubated at 37°C in minimal medium supplemented with a mixture of amino acids prior to plating (Figure 1). There is no measurable increase in the amount of DNA during the first ten-minutes incubation. During this period the synthesis of RNA and protein proceeds. Samples of the culture at proper dilution were plated either on minimal agar medium supplemented with 2.5 percent nutrient broth, or on unsupplemented

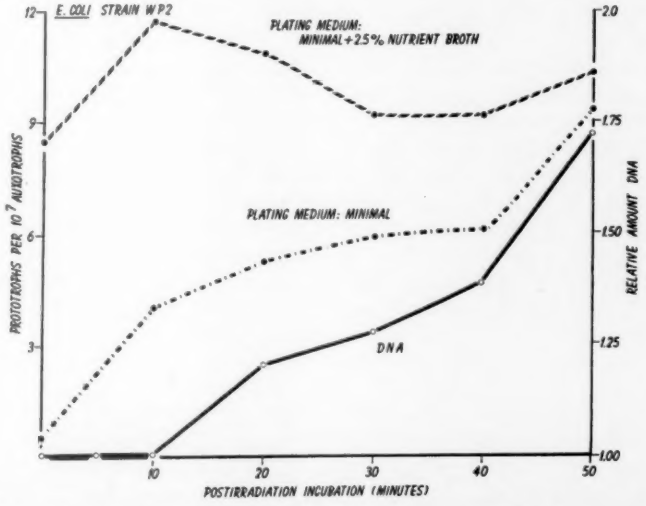

FIGURE 1.—Mutation induction and expression in *E. coli* strain WP2 following exposure to 10 kr X-rays. The culture was incubated, following exposure, in minimal medium supplemented with a mixture of amino acids including the required amino acid, tryptophan, and then plated at appropriate dilution at the indicated times, either on minimal agar medium (mutation expression) or on minimal agar medium supplemented with 2.5 percent Difco nutrient broth (mutation induction). The DNA curve represents the amount of DNA, relative to unincubated controls, which has been formed at the times of plating.

minimal agar medium. The results demonstrate that, in the first ten minutes, an appreciable portion of the total yield of mutations is expressed, as measured on minimal agar medium. These results are quite different from the results observed with UV, where only a very small proportion of the total yield of induced mutations are expressed prior to the initiation of DNA synthesis in the culture (HAAS and DOUDNEY 1959).

Experiments with thymineless, amino acidless polyauxotrophs: Synchronized cultures of strains 15 thy met and 15 thy pro were carefully washed with minimal medium to eliminate contaminative thymine and were then irradiated with 5 or 10 kr of X-rays. The irradiated cells were incubated at 37°C in a medium containing amino acids, including those essential for each strain, but no thymine. The cells were then harvested and plated on minimal medium supplemented with thymine, after washing to remove the amino acids. In the strains tested, a net increase in mutation frequency was observed, compared to that found prior to postirradiation thymineless incubation (Table 1). By measurements before and after incubation, it was determined that measurable net DNA synthesis did not occur during this period. It is to be noted that RNA and protein increase during this period while DNA synthesis is blocked.

Additional studies were carried out with a polyauxotroph requiring thymine, methionine, and tyrosine (Figure 2). The culture was exposed to 10 kr of X-rays, then incubated in medium containing a mixture of amino acids including methionine and tyrosine but no thymine. Samples of this culture were plated, after washing, on minimal agar medium supplemented with methionine and thymine and on minimal agar medium supplemented with methionine, thymine and 2.5 percent nutrient broth. Approximately half of the induced prototrophs (tyr^+) are expressed with 20 minutes incubation. The control cells plated on minimal plus nutrient broth indicate the maximum mutation response observed. An additional 20 minutes incubation in the absence of thymine produced no more expressed prototrophs. This experiment makes it evident that, while approximately half of the prototrophs induced may be expressed in the presence of the required amino acid without the thymine required for DNA synthesis, the other half of the induced prototrophs require thymine, in addition to the amino

TABLE 1

Mutation expression in the absence of thymine in two biauxotrophic strains of Escherichia coli

Strain	Minutes of incubation	Mutation frequency per 10^8 survivors	Relative amount		
			DNA	RNA	Protein
15 thy pro	0	0.5	1.0	1.0	1.0
	20	5.0	1.0	1.4	1.2
	40	3.0	0.9	1.8	1.5
15 thy met	0	1.0	1.0	1.0	1.0
	15	11.0	0.9	1.6	1.2
	30	30.0	1.0	2.1	1.2

The preplating postirradiation incubation medium contained a mixture of amino acids including the amino acid required by the strain (methionine or proline) but no thymine. Plating was on minimal agar medium supplemented with thymine.

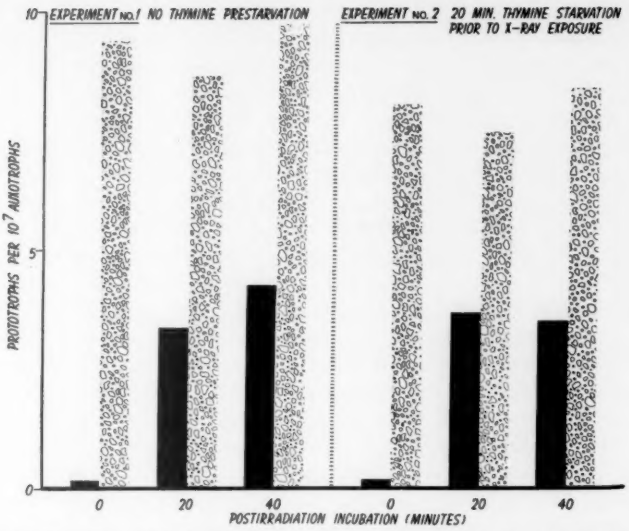

FIGURE 2.—The effect of blockage of DNA synthesis through thymine deprivation on mutation induction and expression in *E. coli* strain 15 thy met tyr, a polyauxotroph requiring thymine, methionine and tyrosine, after exposure to 10 kr X-rays. The tyrosine requirement reversion was followed. The culture was incubated, following exposure, in minimal medium supplemented with a mixture of amino acids but with no thymine added. Samples at appropriate dilution were plated at the indicated times on minimal agar medium supplemented with thymine and methionine (mutation expression, black bars) or minimal agar medium supplemented with thymine, methionine and 2.5 percent Difco nutrient broth (mutation induction, light bars). In Experiment No. 2 the culture was starved for thymine in the above described incubation medium for 20 minutes prior to X-ray exposure.

acid, for expression. In this strain, that proportion of the mutants expressed in the absence of thymine may correspond to that half of the mutants expressed before measurable DNA synthesis in the try strain.

The possibility exists that there is sufficient endogenous thymine retained in the irradiated bacterial cell under these conditions to allow synthesis of the DNA which might be required for mutation induction even in the absence of added thymine. In order to eliminate this possibility, the bacterial culture was starved in medium without thymine for 20 minutes prior to radiation exposure, and the above experiments repeated. As is demonstrated in Figure 2, the results are practically identical to the results obtained in the case of cells not starved for thymine prior to radiation exposure. Approximately one half of the cells are expressed in 20 minutes in absence of thymine. Further incubation in the absence of thymine does not lead to additional expression of the prototrophic phenotype.

Another experiment was carried out along these lines with the same strain. However, in this case, after 40 minutes incubation thymine was added to the

preplating liquid growth medium and both mutation expression and DNA synthesis followed (Figure 3). In this experiment incubation for 20 minutes was sufficient for expression of half of the mutations. Incubation was continued for an additional 20 minutes in absence of thymine, without further expression. The mutation frequency then doubles in the next 20 minutes following the addition of thymine and initiation of DNA synthesis. Figure 3 further demonstrates that there is no measurable net DNA synthesis in 40 minutes in the absence of thymine, but that when the thymine is added, DNA synthesis is initiated in correlation with the increased mutation response. These experiments support the hypothesis that, while approximately one half of the mutations induced by X-rays at this locus can be expressed phenotypically in the absence of DNA synthesis, the other half of the induced mutations require DNA synthesis for expression. The experiments do not eliminate the possibility that thymine derived from degradation of cellular DNA might feed minor new DNA synthesis, thus supporting expression in the absence of added thymine. The role of cellular turnover of DNA in the case of spontaneous mutation in stationary phase cultures has been studied (RYAN 1959). It was finally concluded that the mutations resulted from errors in replication of genetic DNA which was in the process of

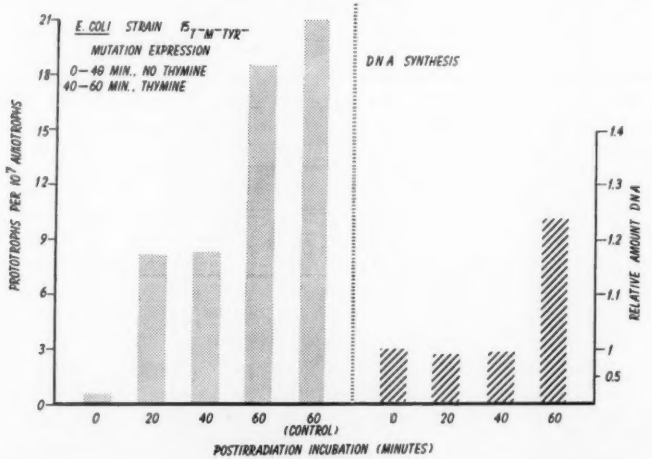

FIGURE 3.—Mutation expression and DNA synthesis in the absence and presence of thymine in *E. coli* strain 15 thy met try, a polyauxotroph requiring thymine, methionine and tyrosine, after exposure to 10 kr X-rays. The tyrosine requirement reversion was followed. The culture was incubated, following exposure, in minimal medium supplemented with a mixture of amino acids, including methionine and tyrosine, the required amino acids, but with no thymine added. At 50 minutes incubation, thymine was added to the culture. Samples at appropriate dilution were plated at the indicated times on minimal agar medium supplemented with methionine and thymine. The 60 minute control was plated on minimal agar medium supplemented with methionine, thymine and 2.5 percent Difco nutrient broth. The DNA values given represent the DNA measured at the times of plating as compared to unincubated controls.

turnover in the nondividing cell. It is very difficult to establish definitive proof which eliminates the last possibility that DNA turnover is involved in the X-ray-induced mutation mechanism which proceeds in the absence of thymine. In our case, the incubation period necessary for mutation expression is very short and normal metabolism is more or less blocked or modified because of the irradiation. Breakdown of nucleic acids and release of mononucleotides into the surrounding medium has been observed with incubation or holding of cells exposed to X-ray, although the nature of the process is not clear. For example, STUY (1960) irradiated certain strains of *E. coli* with high doses of X-rays and observed that about half of the cellular DNA, determined by the diphenylamine method, is lost with incubation. In our case, the dose used is relatively low and the measured amount of DNA remains practically constant during the postirradiation incubation period. If there exists DNA degradation products which are excreted into the medium to provide the thymine necessary to feed DNA synthesis at a low level, they should be eliminated by incubating the cells in a medium which permits degradation of the nucleic acid, followed by washing the cells to remove the surrounding medium containing the degradation products prior to addition of the required amino acid to the culture. Experiments of this type, where the cells are incubated for a period in the absence of the required amino acid to allow any possible degradation of DNA to take place and then washed to remove hypothetical degradation products of DNA, were carried out. This treatment does not prevent the expression of the expected frequency of mutation in the absence of thymine when the required amino acid is added. The results suggest that the synthesis of DNA is blocked in the thymineless strain during thymine-free incubation and that the increase in mutation frequency occurs in the absence of DNA replication.

Thymineless induction of mutation: COUGHLIN and ADELBERG (1956) have described an effect of incubation in the absence of thymine on reversion of the histidineless locus of *E. coli* strain 15 thy his. They found that the frequency of prototrophic cells to viable bacteria increased considerably after incubating the cells in thymine-free synthetic medium. Although the absolute number of prototrophs remained constant during thymine-free incubation, some evidence was reported to eliminate the possibility that differential killing of auxotrophic cells, as compared to prototrophic cells, though thymineless death might account for the observed increase in mutation frequency.

In our experiments with X-ray-induced mutation of the tyrosineless locus in *E. coli* strain 15 thy met tyr the period of incubation in the thymine-free medium is too brief to induce thymineless death. It is improbable therefore that the observed increase in mutation frequency is due to an effect of the thymineless condition comparable to that observed by COUGHLIN and ADELBERG (1956). However, an experiment was undertaken with this strain in order to observe the effect of the thymineless condition on reversion of the *tyr* locus. The organism was incubated in the thymine-free minimal medium containing the required amino acids, methionine and tyrosine, producing considerable "thymineless

inactivation" (Table 2). In spite of the lethal effect, the mutations observed decreased at the same rate as the parent population and the mutation frequency per viable cell remained constant indicating that no change in mutation frequency is produced by the thymineless incubation. It is thus obvious, at least in the case of this strain, that we can ignore any possible contribution of thymineless-induced mutations or of selection of spontaneous prototrophs, through thymineless death, in the studies of X-ray-induced mutation in the thymineless condition. Further it is evident that little or no thymineless induction of mutation is observed at this locus, as was reported by COUGHLIN and ADELBERG (1956) in the case of reversion of the histidine requirement studied by them.

Mutation expression and macromolecular synthesis: HAAS and DOUDNEY (1960) suggested that, with an amino acid deficient strain, the fraction of induced reversions capable of phenotypic expression at a given time following application of the inducting agent can be determined by plating identical samples of the culture on minimal agar medium and on minimal agar medium supplemented with a low level of the required amino acid. It was assumed that the amino acid is necessary for the appearance of the missing enzyme involved in the synthesis of the amino acid required by the strain and controlled by the mutated gene. It was suggested that this technique may serve as a means for differentiating those processes involved in gene replication and mutation from those involved in gene action (i.e., phenotypic expression).

These techniques are utilized in the following studies of expression of X-ray-induced reversion of the try strain. A typically biphasic expression curve is found, as is often observed when the X-ray exposed cells are plated onto minimal agar medium (Figure 4). It is further demonstrated that either chloramphenicol, an antibiotic which blocks protein synthesis, or 6-azauracil, a uracil analog which blocks RNA synthesis as well as protein synthesis, prevents mutation expression. Further studies have demonstrated that holding the irradiated cells at 2°C prevents mutation expression. The absence of an energy source (glucose) or the addition of dinitrophenol also inhibits this phenomenon. The necessity of the essential amino acid, tryptophan, has been demonstrated in these studies.

TABLE 2

Effect of thymine starvation on spontaneous mutation frequency in Escherichia coli *strain 15 thy met tyr*

Minutes of incubation	Percent inactivated	Colony-forming bacteria plated	Average number of tyr^+ colonies found per plate	Frequency of tyr^+ per 10^8 tyr bacteria
0	0	2.4×10^8	14	5.6
30	14.5	2.1×10^8	10	4.9
60	41.7	1.4×10^8	13	9.6
90	68.4	7.6×10^7	3.5	4.6
120	86.2	3.3×10^7	2	4.6

The culture was incubated in minimal medium containing a mixture of amino acids including methionine and tyrosine for the indicated times and then plated on minimal agar medium supplemented with thymine and methionine. As determined by plate washing and replating techniques, no residual divisions of the tyr cells occurred on this medium after plating.

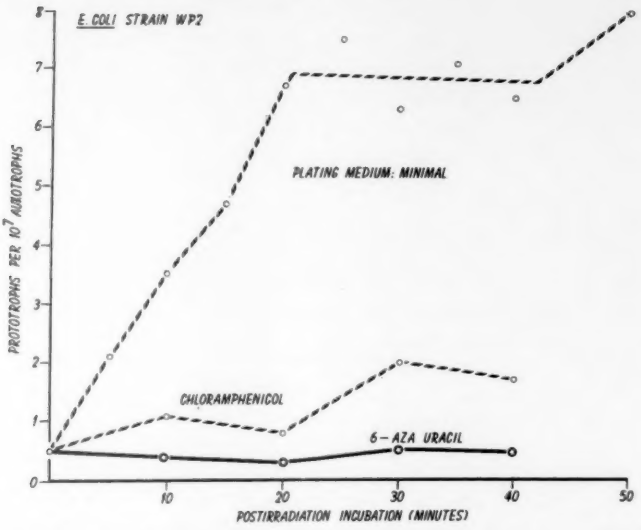

FIGURE 4.—The effect of chloramphenicol and 6-azauracil on mutation expression in *E. coli* strain WP2 following exposure to 10 kr X-rays. The culture was incubated, following exposure, in minimal medium supplemented with a mixture of amino acids including the required amino acid, tryptophan, and then plated at appropriate dilutions on minimal agar medium. The upper curve represents the results obtained in the absence of added antimetabolites while the lower curves represent the effects of 6-azauracil or chloramphenicol, added at initiation of postirradiation incubation, on mutation expression.

It is therefore concluded that the phenotypic expression of the gene modified by X-ray requires RNA and protein synthesis, in order to permit growth on minimal agar medium.

Mutation induction and macromolecular synthesis: It is evident from the above results that, if we are to study mutation *induction* as opposed to expression of the modified phenotypic character, we must by some means provide for those metabolic processes involved in phenotypic expression and requiring the amino acid in which the strain is phenotypically deficient in synthesis. This may be accomplished by plating on minimal agar supplemented with a low level of the amino acid required. In practice, the supplementation of 2.5 percent nutrient broth to the minimal agar medium has proved satisfactory to meet this need.

If we add chloramphenicol at the start of incubation and then incubate for increasing periods of time, no appreciable effect is observed on the frequency of mutations, when the cells are plated on minimal medium supplemented with nutrient broth containing the tryptophan required for expression (Figure 5). On the other hand, it is evident that chloramphenicol interferes with the expression of the mutations as measured by plating on minimal agar medium. Thus, we can conclude that, while most of the potential mutations are not susceptible

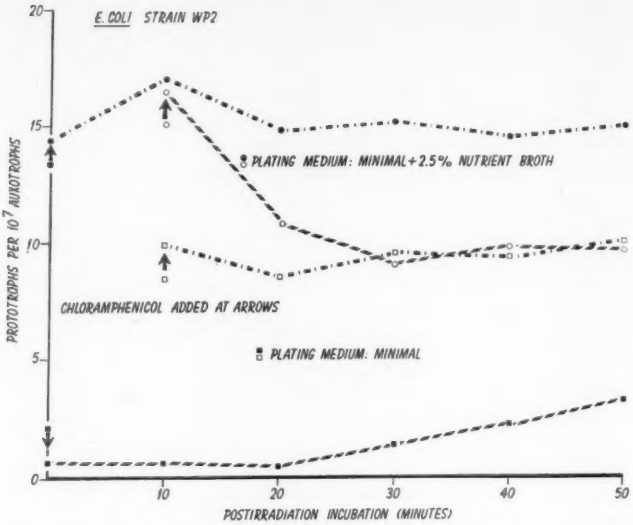

FIGURE 5.—Effect of chloramphenicol on mutation induction and expression in *E. coli* strain WP2 following exposure to 10 kr X-rays. Duplicate cultures were incubated, following exposure, in minimal medium supplemented with a mixture of amino acids including the required amino acid, tryptophan. At the times indicated by the arrows (either 0 time or after ten minutes incubation) chloramphenicol was added to one of the duplicate cultures and incubation continued. Samples were plated on minimal agar medium (mutation expression) or minimal agar medium supplemented with 2.5 percent Difco nutrient broth (mutation induction) at the indicated times.

to chloramphenicol in the sense of being unstable immediately following exposure, their expression is prevented by chloramphenicol. Here again we have a departure from the results with UV where most of the mutations are sensitive to chloramphenicol when this antimetabolite is added immediately (DOUDNEY and HAAS 1958, 1959).

Next, the effect of adding chloramphenicol after a slight period of incubation (ten minutes) was studied. These results make it clear that a period of incubation involving protein synthesis is necessary for the mutations to become *sensitive* to chloramphenicol. Thus, when chloramphenicol is added at ten minutes incubation, about half of the total number of mutations are sensitive to chloramphenicol and are lost through some sort of "mutation frequency decline" process. The other half appear not to be sensitive to chloramphenicol and are not affected by incubation in the presence of chloramphenicol. It is further demonstrated that in the first ten minutes incubation, that half of the mutations which is not affected by chloramphenicol becomes expressed, as measured by plating on minimal agar medium. Thus, we can conclude that the half of the mutations which is not sensitive to chloramphenicol is comparable to that half which is expressed in the first ten minutes in the absence of DNA synthesis, as described

above. Thus after ten minutes incubation following exposure, the population of potential mutations may be divided into two classes rather equal in number: those which are completely expressed and are not sensitive to chloramphenicol and those which are not expressed and are sensitive to chloramphenicol.

Effect of chloramphenicol and 6-azauracil on survival: When either chloramphenicol or 6-azauracil is added immediately after X-ray exposure, a marked lethal effect is observed (Table 3). However, when these antimetabolites are added after ten minutes incubation following radiation exposure this lethal effect is not apparent. Thus, it is evident that, in ten minutes, certain synthetic processes have taken place in repair of the X-ray damage, which leaves the bacterial cell as typically insensitive to any lethal effects of chloramphenicol or 6-azauracil as unirradiated cells. The point to be emphasized, as far as the mutation studies are concerned, is that the changes in the survival produced by these compounds are not correlated with the genetic effects observed. Thus, while the level of survival drops considerably when either chloramphenicol or 6-azauracil is added immediately following radiation exposure (Table 3), the mutation frequency remains constant (Figure 5). On the other hand, with addition of these compounds after ten minutes incubation, the level of survival remains constant while the mutation frequency changes markedly. It is evident therefore that the change in mutation frequency observed with these treatments begun after ten minutes is not merely a reflection of change in survival, nor is the stability of the potential mutations immediately following radiation exposure related to an effect of chloramphenicol or 6-azauracil on the survival of the cells.

Chloramphenicol and 6-azauracil challenge: A correlation between RNA synthesis and "mutation fixation" in UV-irradiated cultures has been previously described (DOUDNEY and HAAS 1959). Since it is apparent that susceptibility of the potential mutation to those treatments leading to lower mutation frequency is lost with increasing periods of postirradiation incubation (WITKIN 1956; DOUDNEY and HAAS 1958), it is evident that blockage to RNA or protein forma-

TABLE 3

Effect of chloramphenicol and 6-azauracil on survival of X-irradiated Escherichia coli *strain WP2*

Minutes of incubation	Colony forming organisms per ml			
	Chloramphenicol		6-azauracil	
	Added at "0" time	Added after 10 minutes	Added at "0" time	Added after 10 minutes
0	1.3×10^8	9.0×10^7	1.2×10^8	1.3×10^8
10	6.1×10^7	8.6×10^7	8.0×10^7	1.2×10^8
20	4.8×10^7	8.3×10^7	8.1×10^7	1.4×10^8
30	2.2×10^7	8.0×10^7	7.5×10^7	1.4×10^8
40	1.6×10^7	9.1×10^7	7.5×10^7	1.3×10^8
50	1.2×10^7	9.8×10^7	6.9×10^7	1.1×10^8
60	1.3×10^7	8.6×10^7	6.5×10^7	1.4×10^8

The irradiated cells, divided into four cultures, are incubated in minimal medium supplemented with a mixture of amino acids including tryptophan. Chloramphenicol or 6-azauracil was added to a selected culture either at "0" time or after ten minutes incubation. Appropriately diluted samples were plated from each culture at the indicated times on minimal agar medium supplemented with 2.5 percent Difco nutrient broth.

tion leads to a decline in mutation frequency *only* provided some significant metabolic event has not taken place. This event we have termed "*mutation fixation.*" Mutation fixation is defined specifically, though arbitrarily, as that biosynthetic event which removes the potential mutation from susceptibility to those treatments which promote the *specific* decline process involved. It should be made clear that mutation fixation is not regarded as the final step in the mutation induction process. Rather, the final step in mutation induction by UV unquestionably appears to be the initial postirradiation synthesis of the genetic complement of DNA.

The time required for mutation fixation can be quantitatively measured by *short term* challenge with chloramphenicol or other agents or conditions which interfere specifically with protein synthesis or RNA synthesis. This challenge procedure demonstrates that fraction of the potential induced mutations "fixed" at any given interval following UV exposure, in the sense that they are not susceptible to the specific mutation frequency decline process. Following the challenge period, which is carried out in liquid medium, plating is on minimal agar medium supplemented with 2.5 percent nutrient broth. This contains

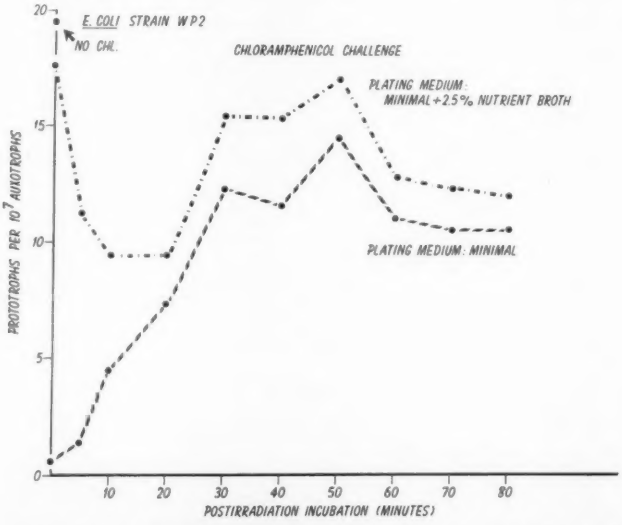

FIGURE 6.—Effect of chloramphenicol challenge on mutation induction and expression in *E. coli* strain WP2 following exposure to 10 kr X-rays. Several identical cultures were incubated, following exposure, in minimal medium supplemented with a mixture of amino acids including the required amino acid, tryptophan. At the indicated times chloramphenicol was added to a selected culture and incubation continued in the presence of chloramphenicol for 50 minutes, a period of time sufficient to eliminate all chloramphenicol sensitive mutations. Following this period of incubation appropriately diluted samples of the cultures were plated on minimal agar medium (mutation expression) or minimal agar medium supplemented with 2.5 percent Difco nutrient broth (mutation induction).

sufficient tryptophan for the protein synthesis necessary for mutation expression, and thus eliminates mutation expression from consideration in the studies.

In Figure 6, a similar study of X-ray-induced mutation with the try strain using essentially the same techniques is demonstrated. The technique consists of adding chloramphenicol after different periods of incubation in minimal medium supplemented with amino acids, including tryptophan, and then continuing the incubation for 50 minutes prior to plating, a period of time sufficient to interfere with all chloramphenicol sensitive mutations. As this figure shows, and in agreement with the above described results, in the first ten minutes half of the mutations become sensitive to chloramphenicol. This sensitivity remains constant for some twenty minutes. Sensitivity to chloramphenicol is rapidly lost with further incubation. Similar results have been obtained using 6-azauracil (Table 4). Thus, it appears that RNA and protein synthesis are involved in the induction of this proportion of the X-ray-induced mutations, as is the case with UV-induced mutations.

The lower curve in Figure 6 demonstrates the results of treating samples of the culture in an identical manner with chloramphenicol, but plating on minimal agar medium instead of minimal medium supplemented with nutrient broth. This curve thus demonstrates only those prototrophs which, at the time of plating, are both expressed and insensitive to the chloramphenicol challenge. It can be seen that during that period in which one half of the mutations become sensitive to chloramphenicol, the other half are rapidly becoming expressed. In addition it is evident that expression of the half of the induced mutations sensitive to chloramphenicol follows the *loss* of sensitivity of these mutations to chloramphenicol.

DISCUSSION

Several recent investigations suggest that UV induces mutation through at least two mechanisms (WITKIN and THEIL 1960; KADA, BRUN and MARCOVICH 1960). In one type of mutation induction process, postirradiation treatments of

TABLE 4

Effect of 6-azauracil challenge on mutation induction and expression in Escherichia coli *strain WP2 following ten kr X-rays*

Time of addition of 6-azauracil (minutes)	Prototrophs per 10^7 survivors	
	Plated on minimal medium plus 2.5 percent nutrient broth	Plated on minimal agar medium
0	28.0	3.7
10	17.4	11.1
20	28.9	22.1
30	33.0	23.7

The X-irradiated cells in several identical cultures were incubated in minimal medium supplemented with a mixture of amino acids including tryptophan. At the indicated times 6-azauracil was added to a selected culture and incubation continued for 50 additional minutes (a period of time sufficient to eliminate all 6-azauracil senstitive mutations). Appropriately diluted samples were then plated on minimal agar medium supplemented with 2.5 percent Difco nutrient broth or on minimal agar medium.

various sorts (and specifically the interruption of RNA or protein synthesis) interfere with mutation induction. With this type of mutation, we may conclude that the initial chemical damage produced by the ultraviolet light is unstable and is subject to modification by postirradiation metabolic processes. It is clear from recent investigations of the mechanism of induction and expression of this type of mutation that while processes upon which mutation induction depends occur prior to DNA synthesis, the final step in mutation induction involves the synthesis of DNA (HAAS and DOUDNEY 1959). The expression of the phenotypic change which is the product of the induced mutation follows the initial post-irradiation synthesis of DNA and is dependent on protein synthesis. It has been suggested that the dependence of mutation expression on protein synthesis following DNA synthesis is related to the appearance of the missing enzyme involved in synthesis of the required amino acid and controlled by the mutated gene in the new DNA (HAAS and DOUDNEY 1959). The other type of UV induced mutation is not influenced significantly by postirradiation metabolic activities, but WITKIN and THEIL (1960) have inferred an involvement of protein synthesis in phenotypic expression of this type of mutation.

A similar division into at least two classes of mutations based on the nature of the induction mechanism appears to hold for X-ray-induced mutation. While a portion of X-ray-induced mutations are influenced by postirradiation conditions, another portion appear to be refractory to these treatments (HAAS and DOUDNEY 1958; KADA, BRUN and MARCOVICH 1960). In the case of the two mutational sites studied here (reversion of the *try* locus in strain WP2 and reversion of the *tyr* locus in strain 15 thy met tyr) the effect of X-ray in inducing mutations seems to be divided rather equally between two mechanisms comparable to those described above. One type of X-ray-induced mutation mechanism seems to be unstable with various postirradiation treatments and dependent on DNA synthesis for induction while the other type appears to be refractory to postirradiation treatments and not to require DNA synthesis for induction.

These data support the hypothesis that the *functional* reversion is produced by X-ray in the gene without the necessity of DNA replication in one half of the X-ray-induced mutations. In experiments with the *try* reversion in strain WP2, incubation for ten minutes with tryptophan was sufficient to permit complete expression of half of the mutations, as measured by plating on minimal agar medium. During this incubation, RNA and protein synthesis proceed, but there is no measurable DNA synthesis. In the case of strain 15 thy met tyr, the tyr^+ reversion took place in the absence of DNA synthesis in thymine-free medium in a comparable time period. It was shown that thymine starvation itself does not cause the increase in mutation frequency observed in this strain and further that endogenous thymine in the cell at the time of radiation does not seem to play any role in the observed increase in mutation frequency. The results make it clear that RNA synthesis and protein synthesis are involved in phenotypic expression of this type of mutation following X-ray exposure.

Since these studies seem to eliminate the possibility of participation of post-

irradiation DNA synthesis in mutation induction for this half of the induced mutations, it may be that the mechanism of action of the ionizing radiation in this case is by direct action on genes *in situ*. Thus, the classical notion of action of radiation in inducing mutations, based on direct hits of the radiation on genetic material, may be still valid for that portion of the mutations induced by ionizing radiation which do not require postirradiation metabolism for induction. It is interesting to note that the target size calculated for induction of reversion in strain WP2 is on the order of 10–30 atoms (KADA, BRUN and MARCOVICH 1960). This was also found to be true for mutation to bacteriophage resistance of *E. coli* strain B/r (DEMEREC and LAJARET 1946). Therefore, it is not difficult to imagine that X-ray might produce mutation through direct physical action at the level of the functional structure of the gene, although the detailed physicochemical aspects of the process are not clear.

The similarity of mechanism of induction of half of the mutations induced by X-ray to those induced by UV is obvious. The increase in mutation frequency as measured by plating on minimal agar medium is dependent on the onset of new DNA synthesis in the case of this half of the X-ray-induced mutations, as was the case with the UV-induced mutations. Mutation expression is complete in both cases before the first postirradiation cell division. Furthermore, in view of the comparable effects of chloramphenicol and 6-azauracil, it is clear that RNA and protein synthesis are involved in the mutation induction process with both types of radiation.

This similarity in the results with the two types of radiation does not necessarily mean an identity of the primary sites of mutation induction. KADA and MARCOVICH (personal communication) have carried out some experiments which favor the hypothesis of difference in primary sites for UV and X-ray-induced mutation. They have irradiated the cells under various physiological conditions which would vary the content of nucleic acids. These observations, in addition to other radiobiological observations, have led them to the supposition that RNA components of the cell contain the site for UV-induced mutation and DNA for X-ray-induced mutation. In the case of X-ray induction of reversion of the *try* locus of strain WP2, it was necessary to incubate ten minutes for the potential mutation to become sensitive to chloramphenicol or 6-azauracil in the case of that half of the mutations which are comparable to UV-induced mutation. This makes it clear that the gaining of *sensitivity* to chloramphenicol or 6-azauracil of the potential X-ray-induced mutation depends on RNA and protein synthesis subsequent to X-ray exposure. The possibility exists that the modification induced by X-ray on some primary site (e.g., on one or both strands of DNA) is transferred, in terms of genetic specificity, to some other material which is sensitive, metabolically, to the addition of 6-azauracil or chloramphenicol with the consequent blockage of RNA or protein synthesis. One possibility is that some specific ribonucleoprotein formed under control of the X-ray-modified DNA carries genetically modified information and that this ribonucleoprotein could then be involved in postirradiation DNA replication in formation of the stable genetic

character. This hypothesis is comparable to the hypothesis proposed for UV-induced mutation by DOUDNEY and HAAS (1959), where some specific RNA modified by incorporation of a UV-modified nucleotide precursor would pass its mutational specificity on to newly formed DNA. These hypotheses are in agreement with a scheme for UV and X-ray-induced mutation, where a convergence of the two mutagenic mechanisms, coming from different primary sites, is supposed (KADA, BRUN and MARCOVICH 1960).

In any case, it is clear that we do not know the primary site or nature of the damage leading either to X-ray or UV-induced mutation. Establishment of the nature of the primary damage in this type of UV or X-ray-induced mutation which is dependent upon DNA replication is required before we can arrive at an understanding of the mechanisms involved in mutation induction. While the studies of HAAS and DOUDNEY (1957) suggest that the potential UV-induced mutation is initially in the form of a mutagenic nucleic acid precursor formed by the action of UV on purine or pyrimidine containing monomers, no conclusive evidence exists to support this hypothesis. The possibility remains that the initial UV damage is to the cellular DNA or to other sites (DOUDNEY and HAAS 1960a). Similar reservations concerning the nature of the primary site of X-ray-induced mutation must be retained.

This investigation makes it clear that one of the mechanisms of X-ray-induced mutation involves DNA replication in the irradiated cells as well as RNA and protein synthesis. The involvement of RNA and protein synthesis in DNA replication following UV exposure has been demonstrated (HAROLD and ZIPORIN 1958; DOUDNEY 1959; DRAKULIC and ERRERA 1959; DOUDNEY and HAAS 1960a,b). The situation in the case of DNA replication following X-ray exposure is undoubtedly more complex though there is some evidence that similar mechanisms may be involved (DOUDNEY 1956). Upon present evidence, it cannot be concluded that DNA is capable of transferring its genetic information to RNA either after X-ray or UV-irradiation, though this possibility must be considered in the evolution of hypotheses for DNA replication and genetic change. Further basic studies are needed to clarify the meaning of the RNA and protein involvement in DNA synthesis in irradiated cells before it will be possible to understand the mechanism of mutation induction.

Recent experiments have shown that cells harvested during that postirradiation period that the potential X-ray-induced mutation is sensitive to chloramphenicol (i.e., between 10 and 20 minutes incubation after X-ray exposure) are much more sensitive, in terms of lethality, to a second period of irradiation with X-ray than at any other phase of postirradiation development of the culture. We would suppose that the X-ray irradiated cells are at a critical phase in the course of repair of their DNA replication mechanism during this period.

SUMMARY

Recent studies have established that various postirradiation treatments specifically affecting RNA or protein synthesis are effective in lowering markedly the

mutation frequency response to ultraviolet light. Several lines of evidence indicate that DNA synthesis is the terminal event in UV-induced mutation, but that phenotypic expression of the genetic change involves protein synthesis subsequent to DNA synthesis. About half of the X-ray-induced reversions of the tryptophan requiring auxotroph, *E. coli* strain WP2, are lost, when the culture is incubated for 50 minutes with chloramphenicol or 6-azauracil, agents which block RNA or protein synthesis. However, unlike UV reversion, it appears that a short period of incubation following X-ray exposure before addition of chloramphenicol or 6-azauracil is necessary for development of the chloramphenicol or 6-azauracil sensitivity of the mutations. These mutations require RNA synthesis, protein synthesis and DNA synthesis for induction in a manner comparable to UV-induced reversion. The other half of the X-ray-induced mutations are not lost when incubated with chloramphenicol or 6-azauracil for 50 minutes prior to plating on tryptophan containing medium. These mutations are completely expressed when plated on minimal medium without tryptophan after a short period of incubation in tryptophan containing medium and before measurable DNA synthesis in the culture occurs. However, expression does not occur in the presence of 6-azauracil or chloramphenicol. In the case of a thymineless, tyrosineless polyauxotroph expression of the tyrosine reversion occurs in the absence of thymine for one half of the mutations while the other half of the mutations requires thymine for expression. The data suggest that with that half of the mutations which can take place in the absence of thymine, the functional reversion is produced by the X-ray in the gene, without the necessity of DNA replication, but that RNA and protein synthesis is required for gene action in phenotypic expression of the reverted character.

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CONTENTS

I

GREGOR MENDEL's letters to CARL NÄGELI. 1866–1873 1

II

DE VRIES, HUGO, Concerning the law of segregation of hybrids 30
CORRENS, C., G. MENDEL's law concerning the behavior of progeny of varietal hybrids 33
TSCHERMAK, E., Concerning artificial crossing in *Pisum sativum* 42

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Turnover-time and production of the planktonic crustacea in limed and reference portion of a bog lake ..... RAYMOND G. STROSS, JOHN C. NEESS and A. D. HASLER

Respiratory energy loss and activity patterns in the meadow vole, *Microtus pennsylvanicus pennsylvanicus* ..... RICHARD G. WIEGERT

The relation of energy requirements of tropical finches to distribution and migration ..... GEORGE W. COX

Population dynamics of some freshwater zooplankton ..... DONALD H. HAZELWOOD and RICHARD A. PARKER

Reproductive ecology and sound production of the toadfish, *Opsanus tau* ..... GRACE-ANN GRAY and HOWARD E. WINN

The contribution of alder trees (*Alnus tenuifolia*) to the primary productivity of Castle Lake, California ..... CHARLES R. GOLDMAN

Some dynamics of spatial distribution within semi-natural populations of prairie deermice ..... C. RICHARD TERMAN

Insect-vegetaiton relationships in an area contaminated by radioactive wastes ..... D. A. CROSSLEY, JR., and HENRY F. HOWDEN

Growth and reproduction of the oyster drill *Eupleura caudata* in the York River, Virginia ..... CLYDE L. MACKENZIE, JR.

The microbiology of Flathead and Rogers Lakes, Montana. II. Vertical distribution of the microbial populations and chemical analyses of their environments ..... LOUISE F. POTTER and GLADYS E. BAKER

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The spruce-fir forests of the Keweenaw Peninsula, Northern Michigan ..... PAUL F. MAYCOCK

Flowering and fruiting in the white oaks. I. Stamanate flowering through pollen dispersal ..... WARD M. SHARP and HENRY H. CHISMAN

Seasonal trends in carbon dioxide absorption, cold resistance, and transpiration of some evergreens ..... JOHNSON PARKER

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CONTENTS OF PRECEDING ISSUES

MARCH, 1961

TANTAWY, A. O., Effects of temperature on productivity and genetic variance of body size in populations of *Drosophila pseudoobscura*.

DAWOOD, M. M., The genetic load in the second chromosomes of some populations of *Drosophila melanogaster* in Egypt.

VAHARU, TIIU, Modification of ultraviolet-induced mutation frequency in *Neurospora crassa*.

EVANS, H. J., Chromatid aberrations induced by gamma irradiation. I. The structure and frequency of chromatid interchanges in diploid and tetraploid cells of *Vicia faba*.

EVANS, H. J., and T. R. L. BIGGER, Chromatid aberrations induced by gamma irradiation. II. Non randomness in the distribution of chromatid aberrations in relation to chromosome length in *Vicia faba* root-tip cells.

MOUTSCHEN, J., Differential sensitivity of mouse spermatogenesis to alkylating agents.

STEINER, ERICH, Incompatibility in the complex-heterozygotes of Oenothera.

PHILLIPS, J. N., JR., Modification of mutation frequency in microorganisms. I. Preirradiation and postirradiation treatment.

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